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THE EFFECT OF DEPTH ON DEVELOPMENT AND SEXUAL DIMORPHISM OF THE SONIC SYSTEM IN DEEP SEA NEOBYTHITINE FISHES: THE UPPER CONTINENTAL SLOPE

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**THE EFFECT OF DEPTH ON DEVELOPMENT AND SEXUAL DIMORPHISM OF THE SONIC
SYSTEM IN DEEP SEA NEOBYTHITINE FISHES: THE UPPER CONTINENTAL SLOPE**

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
at Virginia Commonwealth University

By

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Abstract

Background: Cusk-eels from the subfamily Neobythitinae are one of the major groups of sound-producing fishes on the continental slope. Sounds have never been recorded from a member of this subfamily, and sonic anatomy is considered a proxy for sound production. As the first part of a study on the effects of depth on sonic anatomy, we examined three relatively shallow species from the upper continental slope.

Methods: Three species (*Hoplobrotula armata*, *Neobythites longipes* and *Neobythites unimaculatus*) were examined for sonic anatomy (skeleton, swimbladder and sonic muscles), and sonic and epaxial muscle fibers were measured for diameter. Regressions of dimensions and weights of sonic muscles and swimbladders against fish total length and weight were compared to determine sexual dimorphism and relative development between species.

Results: Four pairs of sonic muscles (two medial and two lateral) originate on the skull and insert on the medial swimbladder or on modified epineural ribs that attach to the lateral swimbladder. The medial and medial intermediate muscles are generally larger in males than females and are made of relatively small fibers (ca 10 μm in diameter), and lateral muscles are generally larger in females and consist of larger fibers as in epaxial muscles. Medial muscles are considerably larger in *Hoplobrotula armata* than in the *Neobythites* species.

Conclusion: Neobythitines from the upper slope have relatively well-developed sexually-dimorphic sonic systems, suggesting that males produce advertisement calls for courtship. There are major quantitative differences between species. We suggest that sound production involves tonic contraction of the large-fibered lateral muscle pair and oscillatory contractions of the

smaller medial fibers setting the swimbladder into vibration. *Hoplobrotula armata* is probably capable of making more intense sounds than the *Neobythies* spp.

1. Introduction

Fishes produce sounds via numerous independently-derived mechanisms (Ladich and Fine, 2006; Parmentier and Diogo, 2006; Fine and Parmentier, 2015), the most ubiquitous of which involves super-fast muscles that drive the swimbladder into vibration (Skoglund, 1961; Connaughton *et al.*, 2000; Connaughton, 2004; Fine *et al.*, 2001; Millot *et al.*, 2011). In the toadfish, for instance courtship boatwhistles with a 200 Hz fundamental frequency require 200 contractions per second from paired sonic muscles intrinsic to the swimbladder (Fine *et al.*, 2001). Furthermore, these muscles can follow an electrical stimulus at 400 Hz without tetanizing. The swimbladder produces sounds as a forced rather than a resonant response (Fine, 2012; Fine and Parmentier, 2015).

Recently slow muscles have been found in carapid fishes (Parmentier *et al.*, 2006b). Rather than completing a twitch in about 10 ms as in the toadfish (Skoglund, 1961; Fine *et al.*, 2001), they require ca 500 ms for a twitch and tetanize at about 10 Hz. The carapid swimbladder can be divided into three regions: the anterior edge for sonic muscle attachment, followed by a stretchable fenestra, and then the remainder of the swimbladder, which is firmly attached to the vertebral column. Sonic muscles from the head pull the anterior swimbladder forward, and sounds are produced during rebound when the swimbladder snaps back to its resting position exciting vibrations of the bladder and the swimbladder plate, a specialized rib that sits over the fenestra. An intermediate condition has been found in glaucosomatid, pempherid and terapontid fishes (Mok *et al.*, 2011; Parmentier *et al.*, 2016) that shares parallels with slow muscle mechanisms, including head muscles that attach to the anterior bladder, a fenestra followed by

firm attachments of the remainder of the bladder to the vertebral column. A glaucosomatid sound pulse has two parts: a weak but rapid pre-pulse indicative of a fast head muscle contraction and a second more intense part caused by rebound of the bladder via a stretched tendon, e.g. a slow type mechanism activated by a fast muscle (Mok *et al.*, 2011).

Little is known about sound production in the deep sea, and early work on slope species by NB Marshall was based on anatomy, e.g. muscles attached to the swimbladder (Marshall, 1962, 1967). Recent passive acoustics studies have concentrated on the continental shelf and upper slope (Mann and Jarvis, 2004; Rountree and Juanes, 2010; Rountree *et al.*, 2012; Wall *et al.*, 2012; Wall *et al.*, 2013; Wall *et al.*, 2014). A potential fish sound has been localized to about 700 m (Mann and Jarvis, 2004), and a long-term installation at similar depths failed to record definite fish sounds (Wall *et al.*, 2014). Owing to the low density of fishes and difficulties of recording on the continental slope, sonic anatomy will remain an important avenue for exploring this aspect of fish biology.

The largely deep-sea family Ophidiidae, related to the carapids with slow muscles, includes two major subfamilies: the Ophidiinae and Neobythitinae (Nielsen *et al.*, 1999)(Fig. 1). The Ophidiinae contains eight genera and about 40 species, and the more speciose Neobythitinae has 38 genera and about 160 species (Nielsen *et al.*, 1999). Sounds have been recorded from only two-shallow water species, both in the Ophidiinae: the striped cusk-eel *Ophidion marginatum* (Mann *et al.*, 1997; Sprague *et al.*, 2001; Rountree and Bowers-Altman, 2002; Mooney *et al.*, 2016;) and *Ophidion rochei* (Kever *et al.*, 2014a; Kever *et al.*, 2014b). Before the discovery of slow muscles, courtship calls of the striped cusk-eel *Ophidion marginatum* provided a riddle because

their peak frequency is above 1 kHz (Mann *et al.*, 1997; Sprague *et al.*, 2001), too high to be produced by rapid muscle contractions. In slow systems muscle contraction determines the number of pulses but not the frequency in the pulse (Parmentier *et al.*, 2010; Mok *et al.*, 2011; Parmentier *et al.*, 2016).

The sonic systems of the Ophidiinae are incredibly complex with atypical antagonistic muscle pairs (ventral, intermediate and dorsal muscles), a neural arch above the first vertebra that can pivot in the anteroposterior plane (the neural rocker), modified anterior ribs including the winglike process and swimbladder plate, a rocker bone (a bean shaped bone protruding from the front of the swimbladder in some but not all species), a swimbladder with a fenestra, and in some cases a posterior ring encasing a membrane, a likely pressure-release system (Rose, 1961; Courtenay, 1971; Parmentier *et al.*, 2006a; Fine *et al.*, 2007; Nguyen *et al.*, 2008; Parmentier *et al.*, 2010; Kever *et al.*, 2012). Recent work has begun to explore the physiology and sounds of *O. rochei*, and surprisingly females but not males appear to have fast sonic muscles (Kever *et al.*, 2014b).

Sounds have never been recorded from any species in the Neobythitinae due to their depths on the continental slope, and the sonic system has been described in a single species *Barathrodemus manatinus* collected between 1800 and 2600 m in the Bahamas (Carter and Musick, 1985). Its body is sexually dimorphic, and females have deeper more robust bodies than males. Carter and Musick describe a ventrolateral sonic muscle in males that originates on the ventrolateral surface of the prootic region of the skull, curves medially and inserts on the medial anterior swimbladder wall. Additionally, a ventromedial muscle from the ventromedial prootic

inserts on wide ribs of the fourth vertebra. The authors commented that the ventromedial muscles are close to the swimbladder but apparently do not contact it. Females have only the ventromedial muscles and could be mute.

The sonic system of *Barathrodemus manatinus* is clearly simpler than any described in the sister subfamily Ophidiinae, which includes occasional species that can live in shallow water near shore (Nielsen *et al.*, 1999). The effects of adaptation to deeper water that include reduced food (Gartner, 1997; Sutton *et al.*, 2010) and increased hydrostatic pressure (Angel, 1997; Bockus and Seibel, 2016) is unknown. As the first part of an effort to investigate changes in the sonic system of neobythitines from shallow to great depths, we describe the sonic system including growth and sexual dimorphism of three relatively shallow water species from the upper continental slope of Southern Taiwan.

2. Materials and methods

Three relatively shallow Neobythitine species *Hoplobrotula armata* (Temminck & Schlegel, 1846), *Neobythites longipes* (Smith & Radcliffe, 1913) and *N. unimaculatus* (Kamohara, 1938) (Fig. 2) were collected from several hundred meter depths. *H. armata* is known to occur from 200-350 m, *N. longipes* from 300-480 m and *N. unimaculatus*, with a broader depth range, from 100-580 m (Masuda *et al.*, 1984; Nielsen *et al.*, 1999; Iwamoto and McCosker, 2014).

The specimens were purchased from the Overseas Chinese Market at the harbor in Donggang Township (120° 26'E, 22°28' N), Pingtung County, Taiwan. These specimens were caught by bottom trawls at the fishing ground adjacent to the waters of Liuqiu island (Township; 120°22'E, 22°22'N, located off the shore of Donggang Township). The fishing ground was on the upper continental slope around 200 – 300 meters. Specimens were fixed in 10% formalin, measured for total length (TL) in cm and weighed in grams. Fish were dissected to expose the swimbladders and sonic muscles, and their origins and insertions were determined. Swimbladders were measured for length and width in mm at their widest point, and muscles were measured for length in mm while still attached in the fish. Muscles were removed, soaked in 0.9% NaCl for 5 min to insure uniform hydration and weighed in mg. Several fish were placed in hot water to remove the tissues and expose the skeleton, which was further cleaned in 3 % hydrogen peroxide.

Quantitative measurements from the swimbladder and sonic muscles were regressed against fish size (muscle and bladder weights against fish weight and linear dimensions against total length). Male and female regressions were compared with analysis of covariance (ANCOVA),

and adjusted means were calculated using regressions for fish of 25 cm TL and 75 g. Regressions were compared between species using ANCOVA although for simplicity we show comparisons of percent muscle length (sonic muscle length index, SMLI) and weight (sonic muscle somatic index, SMSI), calculated respectively by dividing muscle length by fish TL and muscle weight by fish weight with quotients multiplied by 100. SMLI and SMSI for species and sexes were compared by ANOVA followed by Tukey's test.

Sonic and epaxial muscles (one of each muscle per species) were sectioned at 10 μm on a cryostat and stained in methylene blue. Because occasional fibers exhibited a somewhat longitudinal orientation and the tendency of many sonic fibers to be slightly ovular in cross section, we measured the minimum diameter at the widest point of 30 fibers from each muscle and averaged them for an N of 1.

3. Results

Hoplobrotula armata ranged from 14.7 to 29.9 cm TL and from 19.3 to 178.2 g and included 18 males and 15 females (Fig.3). *Neobythites longipes* ranged from 18.7 to 30.1 cm TL and 30.8 to 148.1 g and included 11 males and 12 females. *Neobythites unimaculatus* ranged from 15.3 to 25.4 cm TL and 19.6 to 112.5 g and included 5 males and 8 females. Fish sex was not distinguished externally. All species had similar length-weight regressions in males and females and were combined into a single regression (Fig. 3). Relationship of weight to length follow: *Hoplobrotula armata* $Y = 4.412e^{0.126x}$, $r^2 = 0.93$, *Neobythites longipes* $Y = 3.334e^{0.127x}$, $r^2 = 0.97$ and *Neobythites unimaculatus* $Y = 3.961e^{0.132x}$, $r^2 = 0.97$.

3.1. Skeleton

The skulls of *Hoplobrotula armata*, *Neobythites longipes* and *N. unimaculatus* appear similar and do not exhibit obvious sexual dimorphism. The sonic muscles originate on the intercalarium, prootic and epiotic bones (Fig. 4).

The neural spine of the first vertebra is fixed and shorter than the second. Although somewhat oblique, both have a more vertical orientation than subsequent neural spines. The first three vertebrae possess modified epineural ribs and attach to the lateral edge of the anterior swimbladder (Fig. 5). The ribs are similar in both sexes in *H. armata* (Fig. 5A) and in *N. longipes* (Fig. 2B), but in *N. unimaculatus* the first rib is broader in females (Fig. 5C) than in males (Fig. 5D). The first two ribs expand distally and connect with the third by a thin band of connective tissue to form a united structure (Fig. 5A). In *Hoplobrotula* the first two ribs are slender and curve backward but are somewhat straighter in *N. longipes* and *N. unimaculatus*. In *N. longipes* and *N.*

unimaculatus the ribs are slender proximally and expand laterally to form a structure reminiscent of the winglike process (Fig. 2B, C, D) in the fawn cusk-eel *Lepophidium profundorum* (Fine *et al.*, 2007). The distal tips of these modified ribs connect to the anterolateral edge of the swimbladder. The shorter modified third rib, the swimbladder plate, is attached to the third and fourth vertebrae and is more triangular (Fig. 5A). It extends over the anterodorsal lip of the swimbladder in all three species but is wider in *Hoplobrotula* than in the *Neobythites* spp. In *N. longipes* and *N. unimaculatus* there is a tiny bilobed fan-shaped bony plate on the medial edge of the ventral sides of the third vertebra forward of the swimbladder plate (Fig. 5B). This bone attaches to the anterior dorsal swimbladder forward of the fenestra. The third and fourth vertebrae also contain slender ribs above the swimbladder plate. Ribs on the fourth through 13th vertebrae are thin, angled posteriorly and form a cup around the swimbladder.

3.2. Swimbladder

The swimbladder consists of a single chamber covered with a heavy-white tunica externa (Fig. 6). The bladder lies below the third through 13th vertebrae and is fused to the ventral surfaces of vertebrae 3 through 11. The posterior end of the bladder tapers to a blunt curved tip, which nestles into a concave soft structure that confines the caudal bladder and could function as a shock absorber (Fig. 7). The dorsal midline of the bladder is slightly concave and attaches firmly to the vertebral column and to the ribs dorsolaterally; both form impressions on the bladder surface. In *Holobrotula* the bladder is widest anteriorly, and its lateral extent decreases continuously toward the posterior end. The bladder is relatively deep and circular in cross section. The dorsal edge is straight, but the ventral edge tapers at the rostral and caudal ends. In

both *Neobythites* spp the bladder is more elliptical in cross section and considerably wider than high. The first quarter of the swimbladder forms a neck region that is severely compressed dorsoventrally and then expands somewhat posteriorly, ending in a similar curved tip as in *Hoplobrotula*. Immediately before it starts to taper, in male *Hoplobrotula* the posterior bladder has a tube surrounding a flexible membrane protruding from the ventral surface (Fig. 6A). The tube is absent in females and in the two *Neobythites* species (Fig. 6C, D, 8, 9).

The swimbladder can be divided into three regions: a thick anterior lip, a stretchable fenestra without the heavy white tunica externa, and a posterior region that comprises most of the bladder (Fig. 6B, D). The swimbladder plate is fused to the dorsal surface of the swimbladder anterior to the fenestra, and the lateral edges of the bladder attach to the distal tips of the first two epineural ribs. In *Hoplobrotula* the fenestra extends from right to left edge across the dorsal bladder (Fig. 6). The anterior edge of the fenestra is pigmented, and the posterior edge is a colorless thin membrane. In both *Neobythites* spp. the anterior edge of the fenestra is less colored and extends across the dorsal surface in females but has a narrower side-to-side width in males (Fig. 8B, D). The fenestra has a complex shape like two connected shallow C's: the fenestra curves backward and then forward from both sides of the midline extending laterally (Fig. 8B).

3.3. Sonic Muscles

The three species have four pairs of sonic muscles (Fig. 10, 11), a medial pair (ventral and medial-intermediate) and a lateral pair (lateral, and lateral-intermediate), whose actions would pull the epineural ribs and the anterior swimbladder forward. The muscles originate on posterior

skull bones and insert on the anterodorsal swimbladder and modified epineural ribs, which also attach to the swimbladder (Table 1). All four muscles are present in both sexes, and many are sexually dimorphic (see below). The lower muscles travel below Baudelot's ligament, and the more dorsal intermediate muscles travel above it. Muscles in *Hoplobrotula armata* are larger than in either *Neobythites* species (see below), which are similar albeit with subtle differences.

Ventral muscle (VM)

VM in *Hoplobrotula armata* originates on the rostral interclarium-prootic (Fig. 4), travels below Baudlot's ligament, and inserts broadly on the medial-anterodorsal swimbladder in both sexes (Fig. 10, 12). It is the largest muscle in males. In both *Neobythites*, the smaller VM originates on the rostral interclarium and tapers to a narrow insertion on the medial-anterodorsal swimbladder (Fig. 11, 13). VM in both *Neobythites* spp has a darker color than in *Hoplobrotula armata*.

Medial intermediate muscle (MIM)

MIM in all three species originates behind VM on the caudal intercalarium and inserts on the medial anterodorsal swimbladder above the insertion of VM (Fig. 10, 11, 12, 13). It travels above Baudlot's ligament. MIM is larger in males than in females in all three species but is much smaller in both *Neobythites* than in *Hoplobrotula armata* (see below). Both VM and MIM are darker than the two lateral muscles in *Neobythites*, whereas in *Hoplobrotula armata* all four muscles are similar in color.

Lateral muscle (LM)

In all three species, LM originates on the ventral epiotic and inserts on the anterolateral swimbladder and the distal tip of the ventral face of first and second epineural ribs (Fig. 10, 11, 12, 13). LM is located below Baudlot's ligament and is larger in females than in males (see below).

Lateral intermediate muscle (LIM)

LIM in *Hoplobrotula armata* originates above LM on the dorsal epiotic and travels above Baudlot's ligament. It has a firm insertion on the dorsal side of the distal half of the first and second epineural ribs. In *Neobythites longipes* and *N. unimaculatus*, LIM has a similar origin but a broader insertion dorsally on the distal three quarters of the first two epineural ribs (Fig. 13).

3.4. Swimbladder growth

Swimbladders of all three species grew linearly in length, width and weight with fish size (Supplemental Table 2-4, Fig. 17). With minor exceptions swimbladders were not sexually dimorphic. However, in *Hoplobrotula* the slope of swimbladder width was greater in males ($p = 0.017$) although the adjusted means for a 25 cm fish were relatively similar (17.5 for males and 16.4 for females). In *N. longipes* swimbladders were heavier in males (slope: $p < 0.0001$) and adjusted means for a 75 g fish were 1329 mg for males and 962 mg for females. Increased swimbladder weight is caused by a thicker bladder wall in males than in females. The bladder in *N. longipes* is also thicker than in the other two species. In *N. unimaculatus* bladders were similar in all three measures although caution is necessary due to small sample sizes and different size distributions for each sex.

Despite minor differences in some linear dimensions, the swimbladder linear dimensions and weights were relatively similar in the three species.

3.5. Sonic Muscle Growth

All sonic muscles grew linearly in length and weight in both sexes (Table 2-4, Figure 14, 15, 16).

Hoplobrotula armata

Sonic muscles exhibited minor sexual dimorphism in length of the ventral and medial intermediate muscles but not the lateral two muscles (Table 2, Fig. 14). Slopes of muscle length were equivalent in all muscles, but the intercepts were different in the ventral and medial intermediate muscles. Adjusted means for 25 cm TL males and females respectively were 17.4 and 15.8 mm for VM, 15.5 and 13.1 mm for MIM, 15.1 and 15.2 for LM, and 14.1 and 13.8 for LIM.

Weights for the VM and MIM were considerably greater in males than in females (Table 2, Fig. 14). Adjusted means for 75 g males and females respectively were 119.7 and 67.6 mg for VM and 38.7 and 16.7 mg for MIM. Conversely, LM and LIM were larger in females. Adjusted means were 22.1 and 28.2 mg for LM and 36.1 and 47.4 mg for LIM.

Neobythites longipes

VM and MIM length had similar slopes in males and females (Table 3, Fig. 15), but muscles were somewhat longer in males. For VM adjusted means were 10.7 and 9.4 mm in males and

females respectively. For MIM adjusted means were 9.4 and 8.6 mm. For LM and LIM lengths were similar in both sexes.

Males had heavier VM and MIM than females (Fig. 15). Adjusted means were 23.2 and 1.8 mg for VM and 16.7 and 4.7 mg for MIM. However, LM and LIM were heavier in females. For LM adjusted means were respectively 18.2 and 29.36 mg, and 15.6 and 24.7 for LIM.

Neobythites unimaculatus

Muscles length had similar slopes (VM, MIM, and LM) in males and females (Fig. 16), but LIM was longer in females. Adjusted means were 10.2 mm in males and 12.2 mm in females. Males had heavier VM than females, and adjusted means were 5.6 and 1.1 mg in males and females respectively. However, MIM, LLM and LIM had similar weights in males and females (Table 4, Fig. 16).

3.6. Fiber Diameter

Fiber diameters were measured from a male and female of relatively similar lengths from each species to determine whether qualitative differences exist between sonic and epaxial trunk muscles. In general, medial muscle fibers (VM and MIM) were smaller than in the two lateral muscles (LM and LIM), which were closer in size to epaxial muscle (Table 5, Fig. 20). Although males and females of the same species had similar total lengths, some possible sexual differences may relate to heavier females in the two *Neobythites* species. In *Hoplobrotula armata* VM fibers were approximately double in size in males than females (13.0 to 6.5 μm), and there was no overlap in the ranges. MIM fibers were similar in size with a smaller sexual difference. LM and

LIM diameters were approximately 40 μm in males and were about 50% larger in males than in females. LM and LIM fiber diameters were similar to that of epaxial muscles, which did not vary with sex. Results were similar for *N. longipes* and *N. unimaculatus* except that the LIM in females *N. unimaculatus* had extremely large fibers ($65.4 \mu\text{m} \pm 8.9 \text{ SD}$) and a range of 43 to 78 μm . The large fiber size ($53.5 \pm 9.7 \mu\text{m}$, range 39-76 μm) was confirmed in another female *N. unimaculatus* of 20.1 cm TL and 59.5 g. In conclusion, except for the MIM in female *N. unimaculatus*, medial muscles had small fibers and lateral ones were larger and similar in size to those in trunk muscle.

Comparisons between species

In all species, the percent length of the sonic muscles (sonic muscle length index SMLI) was similar in males and females (Table 6, Fig. 18). Sonic muscles were longer in *Hoplobrotula* than in the two *Neobythites*, which were similar.

The sonic muscle somatic index (SMSI), compared separately in males and females, indicated differences between species (Table 6, Fig. 19). VM was five-fold larger in males and 8-fold larger in females in *Hoplobrotula* than in the two *Neobythites*, which were not different (Fig. 19a). MIM in males was again somewhat larger (2 fold) in *Hoplobrotula armata* than in *Neobythites longipes* and *N. unimaculatus*. Female SMSI was similar in *Hoplobrotula* and *N. unimaculatus*, but *N. longipes* had a smaller muscle (Fig. 19b). LM was similar for all three muscles in males and in females. However, LIM was about two fold larger in *Hoplobrotula* than for the other two in males, but in females it was similar between *Hoplobrotula armata* and *Neobythites unimaculatus* and smaller in *Neobythites longipes*.

4. Discussion

The Neobythitinae, a group of deep-water fishes found largely on the continental slope, comprise the largest subfamily in the family Ophidiidae (Nielsen *et al.*, 1999). Sounds have not been recorded from neobythitines, and sonic anatomy has been described only for *Barathodemus manatinus*, a deep-water species (1800-2600 m) in the Atlantic (Carter and Musick, 1985). Here, we examined three species from the upper continental slope (relatively shallow species) for later comparison with deeper species down to 5 km. Because of difficulties in recording deep-water fishes, anatomy is taken as a proxy for the ability to produce sounds (Marshall, 1962, 1967; Fine and Parmentier, 2015).

The swimbladders of the three neobythitines are separated into three regions: an anterior lip, followed by a thin stretchable fenestra without a heavy external covering tunic, and a posterior region that is firmly attached to the ventral surface of the vertebral column and ribs. Anteriorly, the first three specialized epineural ribs are united by connective tissue and attach to the lateral edge of the swimbladder. The third rib, the swimbladder plate, also attaches to the dorsal surface of the swimbladder medially forward of the plate. Posterior to the bladder is a soft concave structure that mates with the posterior contour of the bladder and appears to help anchor it in place. To our knowledge this structure has not been described previously. Male *Hoplobrotula armata* have a posterior tube on the ventral swimbladder that is not present in females or in the other two species. A more elaborate tube that penetrates the interior of the bladder also occurs in male *Ophidion rochei* (Ophidiinae) (Parmentier *et al.*, 2010). Finally a dorsoventrally compressed neck region occurs in the anterior portion of the swimbladder in both

Neobythtine spp. The swimbladder of *N. longipes* is heavier than bladders of the other species due to thicker walls.

The three species have four sonic head muscles, a medial and a lateral pair. The two lower muscles travel below Baudelot's ligament, and the two upper ones above. The lower ventral (VM) and more dorsal medial intermediate muscles (MIM) originate, with some variation, on the prootic and intercalarium of the neurocranium and insert respectively lower and higher up on the dorsoanterior lip of the medial swimbladder. The more lateral pair, the lateral (LM) and lateral intermediate muscles (LIM) originate on the epiotic and insert on the first two epineural ribs. The lateral muscle but not the lateral intermediate muscle contacts the swimbladder directly. LM inserts on the distal tip of the dorsal ribs, and LIM inserts more broadly on the ventral side of the distal halves of the two ribs. In the two *Neobythites* species, the VM and MIM are darker in color than the other sonic muscles. Notably VM and MIM have small-diameter fibers (ca 10 μm), whereas LM and LIM have larger diameters that are were similar to those in epaxial trunk muscle. One surprising exception is that MIM in *N. unimaculatus* also has large- diameter fibers, suggesting a high degree of evolutionary plasticity in this muscle.

There are major differences between sonic muscles in these shallow species and in the deeper-water *Barathodemus manatinus* (Carter and Musick, 1985), which has only two, apparently reduced, muscles in males and one in females. Both muscles originate on the prootic, and the ventrolateral sonic muscle, with a more lateral origin, curves medially and inserts on the anterior swimbladder wall in males. The ventromedial sonic muscle in males and females has a more medial origin and inserts broadly on the distal surface of specialized ribs on the 4th vertebra.

The authors do not mention a swimbladder fenestra. Since no muscles originated on the epiotic, it is likely that these two muscles are homologous to the medial pair of muscles in the shallow species and that the insertion of one of the muscles has migrated to a more lateral position. Findings from *B. manatinus* supports the hypothesis that sonic systems are reduced in deep-water neobythitines.

4.1. Sexual dimorphism and muscle size

VM and MIM are slightly longer in male than in female *H. armata* and *N. longipes*. Further, these muscles are longer in *H. armata* in both males and females than in the two *Neobythites* species, which are similar. Relative length likely reflects minor difference in body proportion and is unlikely to have major consequences for sound production. However, major differences occurred in muscle weight, which would affect swimbladder vibration. VM and MIM are larger in males in *H. armata* and *N. longipes*. Sexual differences appear similar in *N. unimaculatus* although it is less clear due to small sample sizes with larger females and smaller males. LM and LIM are larger in females in *H. armata* and *N. longipes* and were suggestive in *N. unimaculatus*.

Fiber diameters in VM are larger in males than in females in all three species as is MIM in *H. armata* and *N. longipes*. Female *N. unimaculatus* had exceptionally large in MIM fibers that were equivalent in size to the lateral sonic and epaxial muscles. LM and LIM fibers appear larger in *H. armata* males and in females for *N. longipes* and *N. unimaculatus* although some of these differences may relate to the heavier female sampled. Similarly, in *Ophidion barbatum*, the intermediate muscle fibers are larger than in epaxial muscle (Parmintier *et al.*, 2006a).

VM is considerably heavier in *H. armata* than in either *Neobythites* species in both sexes, and MIM is larger in males but overlaps in size with *N. unimaculatus* females. LM is similar in both sexes in all species, and LIM is again larger in male *H. armata*. In females LIM overlaps with *N. unimaculatus* and both are larger than in *N. longipes*. Larger medial muscles in *H. armata* than in the two *Neobythites* spp. indicates a variation similar to that in some sciaenids: spot *Leiostomus xanthurus* have reduced muscles, and Atlantic croaker *Micropogonias undulatus* and weakfish *Cynosion regalis* have much larger ones and produce louder sounds (Hill *et al.*, 1987). Thus there are likely to be major differences in sonic behavior and abilities among species in upper slope habitats.

With one exception, fiber diameters are similar across species with smaller fibers in medial and larger ones in lateral muscles, which are similar in size to those in trunk muscle. Sonic muscle fibers tend to have small diameters in many fishes (Fine and Parmentier, 2015), an adaptation that increases surface area favoring increased transport of oxygen and carbon dioxide in and out of the fiber and transport of ATP within the fiber since mitochondria tend to have restricted subcellular distributions. Notably sonic fibers in the oyster toadfish are smaller in males although males have larger sonic muscles (Fine *et al.*, 1990; Fine *et al.*, 1993). Large muscle fibers, as in trunk muscles, have more room for myofibrils, an adaptation to increase contraction power (Rome and Lindstedt, 1998). Lateral muscles in the neobythitines pull epineural ribs and attached swimbladder forward and should be capable of maintaining greater force than the medial muscles.

4.2. Function

Coupled with findings in cusk-eels in the subfamily Ophidiinae (Mann *et al.*, 1997; Parmentier *et al.*, 2010; Rountree *et al.*, 2012; Kever *et al.*, 2012; Kever *et al.*, 2014b; Mooney *et al.*, 2016) larger medial muscles in males suggest that the three *neobythines* fishes produce male advertisement calls to attract females for mating. Also, males can likely produce more intense sounds than females.

Swimbladder fenestras occur in species with slow muscles (*Carapus* and likely many ophidiids (Fine *et al.*, 2007; Nguyen *et al.*, 2008; Parmentier *et al.*, 2010; Parmentier *et al.*, 2006a; Parmentier *et al.*, 2006b) and fast muscles (glaucosomatids, pempherids and terapontids) (Mok *et al.*, 2011; Parmentier *et al.*, 2016). Interestingly female but not male *Ophidion rochei* have fast sonic muscles (Kever *et al.*, 2014b). Based on differences in muscle fiber diameter and parallels with findings in *Ophidion rochei* (Parmentier *et al.*, 2010; Kever *et al.*, 2014a; Kever *et al.*, 2014b;), we suggest that during sound production, the lateral pairs of muscles with large fibers contract tonically pull on the epineural ribs, extending the lateral edges of the swimbladder. The medial muscles with smaller fibers will likely oscillate causing a sound cycle or a sound pulse with each contraction that pulls the medial swimbladder forward. The larger VM and MIM and possibly the posterior tube (Parmentier *et al.*, 2010) in *H. armata* suggest that it is capable of making more intense sounds than the *Neobythites* spp. Conversely the darker muscles, suggesting more mitochondria (Appelt *et al.*, 1991) in the *Neobythites* spp, which may be more fatigue resistant and produce sounds for a longer period.

4.3. Conclusions

The sonic system of neobythitine fishes on the upper slope is well-developed although there are quantitative differences between species. There are two medial and two lateral pairs of extrinsic sonic swimbladder muscles. The medial muscles are larger in males and made of small fibers, also larger in males (with one exception). Lateral muscles tend to be larger in females, and fiber diameter is similar to those in epaxial trunk muscles. Lateral muscle contraction extends specialized epineural ribs and the lateral swimbladder, and medial fibers will cause oscillation of the anterior swimbladder wall. Sexual dimorphism suggests that males produce advertisement calls to attract females for mating. Comparisons with *Barathodemus manatinus* suggests that the system is reduced in species on the lower continental slope.

Table 1 Summary of origins and insertions of four sonic muscles in *Hoplobrotula armata* and *Neobythites spp.*

		<i>Hoplobrotula armata</i>	<i>Neobythites species</i>
VM	Origin	Rostal Interclarium-Prootic	Rostal Interclarium
	Insertion	Medial anterodorsal SB	Medial antero-dorsal SB
MIM	Origin	Caudal Interclarium	Caudal Interclarium
	Insertion	Medial anterodorsal SB above VM	Medial anterodorsal SB above VM
LM	Origin	Epiotic	Epiotic
	Insertion	Anterolateral corner of SB, distal tip ventral face of 1st and 2nd epineural ribs	Anterolateral SB, distal tip ventral face of 1st and 2nd epineural ribs
LIM	Origin	Epiotic	Epiotic
	Insertion	Distal half of dorsal face of 1st and 2nd epineural ribs	Distal three quarters of dorsal 1st and 2nd epineural ribs

Table 2 *Hoplobrotula armata*: regression equations of sonic muscles weight and length, gonad G weight, and swimbladder SB weight and length against fish weight and length, coefficients of determination, analysis of covariance and adjusted means for a 75 g and 25 cm total length fish for males M and females F. Abbreviations: VM: ventral muscle, MIM: medial-intermediate muscle, LM: lateral muscle, LIM: lateral-intermediate muscle.

structure	Sex	n	Regression equation	r ²	p -value	Slope		Intercept		Adj. Mean
						F	p -value	F	p -value	
Weight										
VM	M	18	Y = -35.94 + 2.075X	0.9026	<0.0001	F _{1,29} = 38.81	<0.0001	a	a	119.7
	F	15	Y = 8.259 + 0.7910X	0.7764	<0.0001					67.6
MIM	M	18	Y= -11.89 + 0.6145X	0.8481	<0.0001	F _{1,29} = 25.75	<0.0001	a	a	38.7
	F	15	Y = 0.3521 + 0.2176X	0.6504	0.0003					16.7
LM	M	18	Y = 1.274 + 0.2782 X	0.7515	<0.0001	F _{1,29} = 6.8699	0.0138	a	a	22.1
	F	15	Y = -7.899 + 0.4815X	0.8077	<0.0001					28.2
LIM	M	18	Y = 11.47 + 0.3284X	0.5285	0.0006	F _{1,29} = 14.3411	0.0007	a	a	36.1
	F	15	Y = -7.289 + 0.7294X	0.8881	<0.0001					47.4
G	M	15	Y = -35.89 + 0.5431X	0.5549	0.0014	F _{1,25} = 1.2305	0.2779	F _{1,26} = 28.7785	<0.0001	4.8
	F	14	Y = -10.51 + 0.9670X	0.4463	0.0090					62.0
SB	M	18	Y = - 62.81 + 16.12X	0.9277	<0.0001	F _{1,29} = 1.0275	0.3191	F _{1,30} = 1.3383	0.2565	1146
	F	15	Y = 63.03 + 13.70X	0.7731	<0.0001					1091
Length										
VM	M	18	Y = - 10.34 + 1.110X	0.8399	<0.0001	F _{1,29} = 12.6684	0.0013	a	a	17.4
	F	15	Y = 0.0961 + 0.6290X	0.8712	<0.0001					15.8
MIM	M	18	Y = - 9.578 + 1.004X	0.8411	<0.0001	F _{1,29} = 6.6894	0.0149	a	a	15.5
	F	15	Y = -1.777 + 0.6308X	0.7815	<0.0001					13.1
LM	M	18	Y = - 3.438 + 0.7412X	0.5953	0.0002	F _{1,29} = 0.2730	0.6053	F _{1,30} = 0.0695	0.7938	15.1
	F	15	Y = -1.064 + 0.6484X	0.7793	<0.0001					15.2
LIM	M	18	Y = - 3.017 + 0.683X	0.5411	0.0005	F _{1,29} = 0.1836	0.6715	F _{1,30} = 0.0493	0.8257	14.1
	F	15	Y = - 1.229 + 0.6025X	0.7128	<0.0001					13.8
SBL	M	18	Y = -1.609 + 1.868X	0.7650	<0.0001	F _{1,29} = 0.4719	0.4976	F _{1,30} = 1.2345	0.2754	45.1
	F	15	Y = 1.771 + 1.686X	0.9577	<0.0001					43.9
SBW	M	18	Y= - 10.76 + 1.132X	0.8891	<0.0001	F _{1,19} = 6.8286	0.0171	a	a	17.5
	F	15	Y= - 3.073 + 0.7772X	0.9428	<0.0001					16.4

a: Because the slopes differ much and it was not possible to test whether the intercepts differ significantly.

Table 3 *Neobythites longipes*: regression equations of sonic muscles weight and length, gonad G weight, and swimbladder SB weight and length against fish weight and length, coefficients of determination, analysis of covariance and adjusted means for a 75 g and 25 cm total length fish for males M and females F. Abbreviations: VM: ventral muscle, MIM: medial-intermediate muscle, LM: lateral muscle, LIM: lateral-intermediate muscle.

structure	Sex	n	Regression equation	r^2	p -value	Slope		Intercept		Adj. Mean
						F	p -value	F	p -value	
Weight										
VM	M	11	Y = - 1.129 + 0.3246 X	0.9037	<0.0001	$F_{1,19} = 79.3714$	< 0.0001	a	a	23.2
	F	12	Y = - 1.870 + 0.0469X	0.8685	<0.0001					1.6
MIM	M	11	Y = 0.4765 + 0.2167X	0.7681	0.0004	$F_{1,19} = 13.5603$	0.0016	a	a	16.7
	F	12	Y = 0.5351 + 0.0549X	0.3659	0.0372					4.7
LM	M	11	Y = 0.4902 + 0.2354X	0.8474	<0.0001	$F_{1,19} = 15.5135$	0.0009	a	a	18.2
	F	12	Y = - 4.389 + 0.4500X	0.9325	<0.0001					29.4
LIM	M	11	Y = 6.635 + 0.1190X	0.4521	0.0234	$F_{1,19} = 9.2516$	0.0067	a	a	15.6
	F	12	Y = - 2.935 + 0.3681X	0.7895	0.0001					24.7
G	M	11	Y = - 10.30 + 0.7013X	0.8228	0.0001	$F_{1,19} = 1.3448$	0.2605	$F_{1,20} = 3.6809$	0.0694	42.3
	F	12	Y = - 155.5 + 13.03X	0.1804	0.1687					821.8
SB	M	11	Y = - 75.24 + 18.72X	0.9769	<0.0001	$F_{1,19} = 25.0728$	<0.0001	a	a	1328.8
	F	12	Y = - 19.57 + 13.09X	0.9773	<0.0001					962.2
Length										
VM	M	11	Y = 2.282 + 0.3378X	0.8575	<0.0001	$F_{1,19} = 0.3022$	0.5889	$F_{1,20} = 36.6983$	<0.0001	10.7
	F	12	Y = 0.0745 + 0.3735X	0.8714	<0.0001					9.4
MIM	M	11	Y = 2.705 + 0.2663X	0.7240	0.0009	$F_{1,19} = 1.8061$	0.1948	$F_{1,20} = 17.9871$	0.0004	9.4
	F	12	Y = - 0.3810 + 0.3591X	0.8738	< 0.0001					8.6
LM	M	11	Y = 2.657 + 0.3967X	0.8612	<0.0001	$F_{1,19} = 0.9867$	0.333	$F_{1,20} = 0.0226$	0.8819	12.6
	F	12	Y = 0.4954 + 0.2522X	0.8165	< 0.0001					6.8
LIM	M	11	Y = 2.355 + 0.3826X	0.6242	0.0038	$F_{1,19} = 0.0372$	0.849	$F_{1,20} = 0.8115$	0.3784	11.9
	F	12	Y = 2.184 + 0.4041X	0.8289	< 0.0001					12.3
SBL	M	11	Y = - 8.181 + 2.006 X	0.9863	<0.0001	$F_{1,19} = 0.3512$	0.5604	$F_{1,20} = 0.3634$	0.5534	42.0
	F	12	Y = -10.94 + 2.112 X	0.9516	<0.0001					41.9
SBW	M	11	Y = - 5.794 + 0.7860X	0.7210	0.0009	$F_{1,19} = 0.2051$	0.6558	$F_{1,20} = 0.0203$	0.8881	13.9
	F	12	Y = - 3.346 + 0.6736X	0.5788	0.0041					13.5

a: The slopes differ much and it was not possible to test whether the intercepts differ significantly.

Table 4 *Neobythites unimaculatus*: regression equations of sonic muscles weight and length, gonad G weight, and swimbladder SB weight and length against fish weight and length, coefficients of determination, analysis of covariance and adjusted means for a 75 g and 25 cm total length fish for males M and females F. Abbreviations: VM: ventral muscle, MIM: medial-intermediate muscle, LM: lateral muscle, LIM: lateral-intermediate muscle.

structure	Sex	n	Regression equation	r^2	p -value	Slope		Intercept		Adj. Mean
						F	p -value	F	p -value	
Weight										
VM	M	5	Y = - 0.7849 + 0.0853X	0.7685	0.0510	$F_{1,9} = 4.2624$	0.0689	$F_{1,10} = 26.5041$	0.0005	5.6
	F	8	Y = - 0.9299 + 0.0268X	0.4553	0.0664					1.1
MIM	M	5	Y = - 5.428 + 0.2187X	0.4339	0.2267	$F_{1,9} = 1.7741$	0.2156	$F_{1,10} = 0.0616$	0.8091	11.0
	F	8	Y = 4.675 + 0.0504X	0.1079	0.4269					8.5
LM	M	5	Y = 6.829 + 0.1227X	0.3497	0.2936	$F_{1,9} = 4.0707$	0.0744	$F_{1,10} = 1.9109$	0.1969	16.0
	F	8	Y = - 6.945 + 0.4832X	0.8287	0.0017					29.3
LIM	M	5	Y = 3.821 + 0.1082X	0.3611	0.2839	$F_{1,9} = 0.1559$	0.7021	$F_{1,10} = 3.6094$	0.0866	11.9
	F	8	Y = 8.009 + 0.2210X	0.2652	0.1916					24.6
G	M	5	Y = 3.642 + 0.2529X	0.2958	0.3434	$F_{1,9} = 0.0388$	0.8482	$F_{1,10} = 7.0392$	0.0242	22.6
	F	8	Y = 448.3 – 1.200X	0.0152	0.7713					358.3
SB	M	5	Y = 26.97 + 13.86X	0.8958	0.0148	$F_{1,9} = 0.8029$	0.3936	$F_{1,10} = 0.5854$	0.4619	1066.5
	F	8	Y = 137.5 + 10.87X	0.8952	0.0004					952.8
Length										
VM	M	5	Y = 1.912 + 0.2611X	0.7877	0.0445	$F_{1,9} = 0.1403$	0.7167	$F_{1,10} = 1.1104$	0.3168	8.4
	F	8	Y = 0.6700 + 0.3570X	0.3927	0.0964					9.6
MIM	M	5	Y = - 0.0751 + 0.3330X	0.9364	0.0069	$F_{1,9} = 0.0061$	0.9395	$F_{1,10} = 1.7015$	0.2213	8.3
	F	8	Y = 1.395 + 0.3092X	0.2479	0.2093					9.1
LM	M	5	Y = 1.422 + 0.3905X	0.4103	0.2443	$F_{1,9} = 0.2225$	0.6484	$F_{1,10} = 3.2189$	0.103	11.2
	F	8	Y = 0.1450 + 0.5130X	0.7359	0.0064					13.0
LIM	M	5	Y = 2.996 + 0.2892X	0.9113	0.0115	$F_{1,9} = 3.3998$	0.0983	$F_{1,10} = 6.2493$	0.0315	10.2
	F	8	Y = - 0.5668 + 0.5110X	0.8635	0.0008					12.2
SBL	M	5	Y = - 0.7787 + 1.903 X	0.9140	0.0110	$F_{1,9} = 0.3711$	0.5575	$F_{1,10} = 2.7644$	0.1274	46.8
	F	8	Y = 6.638 + 1.629 X	0.8525	0.0011					47.4
SBW	M	5	Y = - 4.778 + 0.8206X	0.8039	0.0393	$F_{1,9} = 1.0586$	0.3304	$F_{1,10} = 0.1126$	0.7441	15.7
	F	8	Y = 3.092 + 0.4101X	0.2890	0.1694					13.3

a: The slopes differ much and it was not possible to test whether the intercepts differ significantly.

Table 5 Mean \pm SD and range in minimal fiber diameter for sonic and epaxial muscles from an individual male and female in three neobythitines. Abbreviations: VM ventral sonic muscle, MIM medial intermediate sonic muscle, LM lateral sonic muscle, LIM lateral intermediate sonic muscle, and EPX epaxial muscle. The total length and weight for each species are included.

<i>Hoplobrotula armata</i>										
	VM _m	VM _f	MIM _m	MIM _f	LM _m	LM _f	LIM _m	LIM _f	EPX _m	EPX _f
$\bar{x} \pm SD$	13.0 \pm 1.3	6.5 \pm 1.5	12.8 \pm 1.4	9.1 \pm 1.5	41.7 \pm 4.0	27.5 \pm 6.1	36.2 \pm 4.2	21.6 \pm 3.4	38.4 \pm 9.1	37.6 \pm 10.3
Range	11 - 16	4 - 10	10 - 15	7 - 13	35 - 50	16 - 40	29 - 45	17 - 32	24 - 70	24 - 66
Weight _m	143.7 g	Weight _f	150.8 g	TL _m	26.2 cm	TL _f	28.4 cm			
<i>Neobythites longipes</i>										
$\bar{x} \pm SD$	12.4 \pm 1.2	7.6 \pm 1.2	10.4 \pm 1.5	8.3 \pm 1.9	38.4 \pm 4.4	45.9 \pm 5.2	40.9 \pm 5.7	49.0 \pm 6.1	51.4 \pm 8.0	72.6 \pm 9.4
Range	10 - 15	5 - 10	8 - 13	6 - 11	32 - 46	37 - 58	30 - 56	40 - 68	38 - 66	52 - 89
Weight _m	55.2 g	Weight _f	86.5 g	TL _m	23.0 cm	TL _f	25.4 cm			
<i>Neobythites unimaculatus</i>										
$\bar{x} \pm SD$	13.8 \pm 1.9	8.3 \pm 1.0	11.7 \pm 1.7	65.4 \pm 8.9	39.6 \pm 5.2	57.2 \pm 8.1	37.1 \pm 4.8	48.7 \pm 7.0	50.2 \pm 13.0	60.9 \pm 7.8
Range	10 - 18	6 - 10	8 - 17	43 - 78	32 - 49	46 - 81	29 - 45	31 - 65	32 - 75	43 - 74
Weight _m	45.5 g	Weight _f	56.5 g	TL _m	19.5 cm	TL _f	19.6 cm			

Table 6 Mean±SE sonic muscle length index (SMLI) and somatic muscle somatic index (SMSI) for three neobythitine species

	<i>H. armata</i>		<i>N. longipes</i>		<i>N. unimaculatus</i>	
	Male	Female	Male	Female	Male	Female
	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$
SMLI						
VM	6.7±0.19	6.3±0.1	4.4±0.1	3.8±0.1	3.6±0.1	3.9±0.2
MIM	6.0±0.2	5.5±0.2	4.0±0.1	3.4±0.1	3.3±0.04	3.7±0.6
LM	6.0±0.2	6.0±0.2	5.2±0.1	5.1±0.1	4.7±0.3	5.2±0.1
LIM	5.5±0.2	5.5±0.2	4.0±0.1	5.0±0.1	4.5±0.1	4.9±0.1
SMSI						
VM	0.160±0.01	0.088±0.005	0.030±0.002	0.002±0.0003	0.007±0.001	0.005±0.004
MIM	0.046±0.003	0.023±0.002	0.023±0.002	0.006±0.001	0.010±0.003	0.023±0.008
LM	0.030±0.002	0.040±0.002	0.024±0.001	0.038±0.003	0.028±0.004	0.100±0.48
LIM	0.048±0.003	0.066±0.003	0.025±0.002	0.032±0.002	0.020±0.003	0.090±0.043

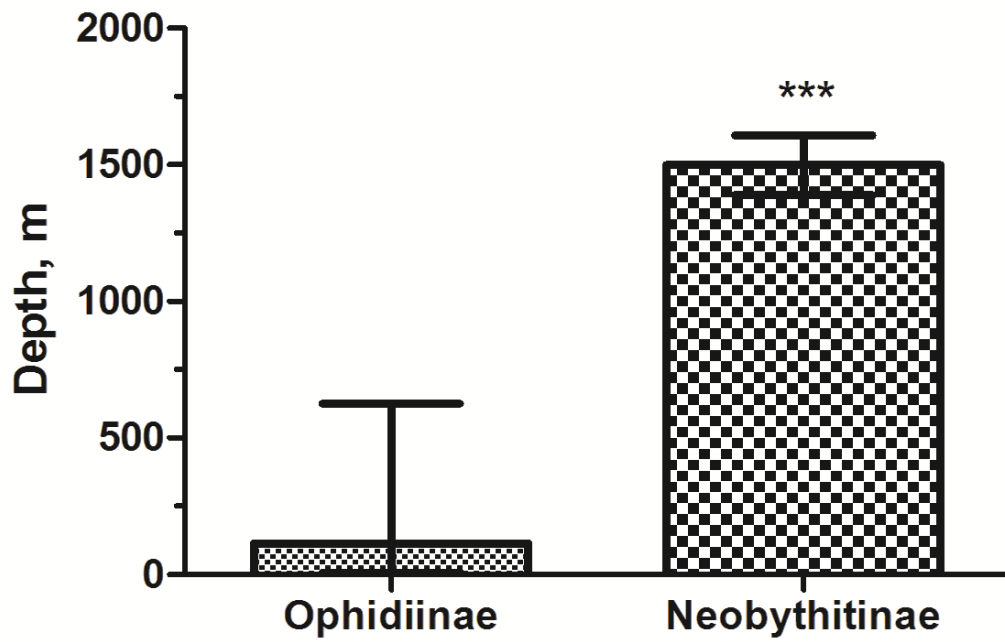


Figure 1 Depth in meters (Mean \pm SE) for species in the subfamilies Ophidiinae and Neobythitinae. Data from Nielsen (1999): $t_{189} = 6.51$, $P < 0.0001$.

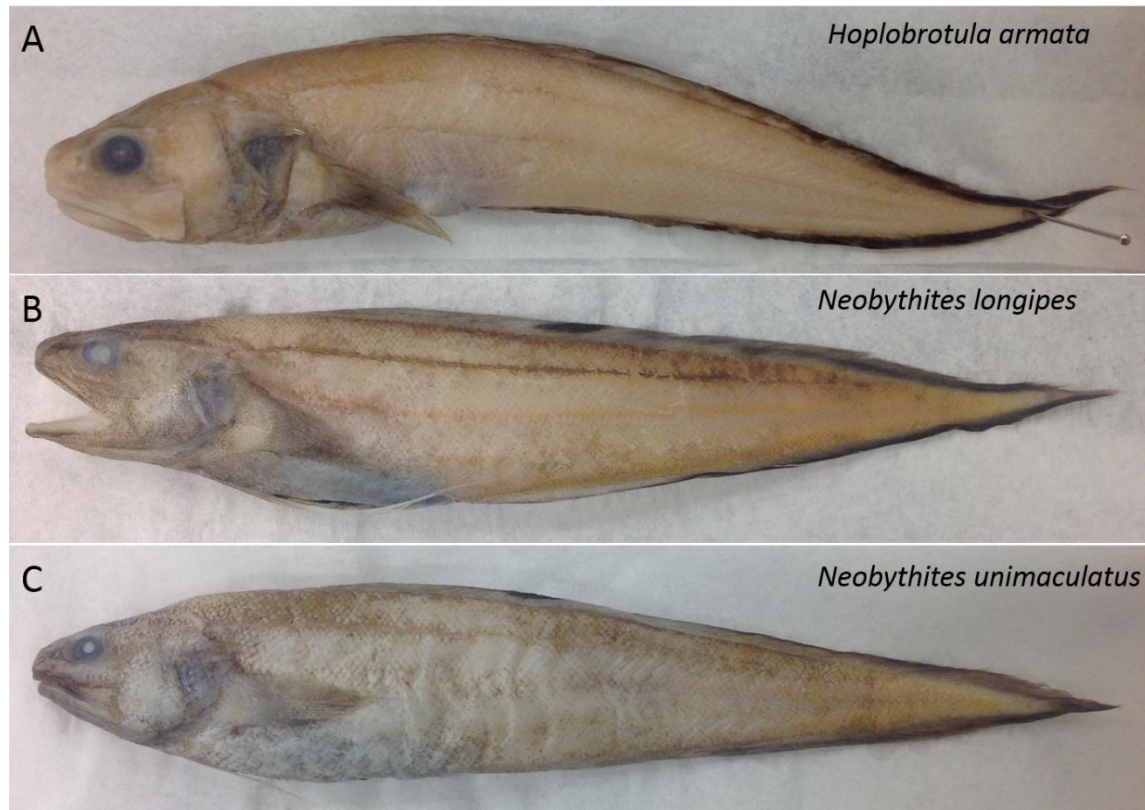


Figure 2 Photographs of (a) *Hoplobrotula armata*, (b) *Neobythites longipes* and (c) *Neobythites unimaculatus*. *H. armata* has continuous black dorsal and anal fins. Both *Neobythites* spp. have a black spot on the dorsal fin, but *N. longipes* has long pelvic fins that extend caudal to the anus, and *N. unimaculatus* has shorter pelvic fins that terminate before the anal opening.

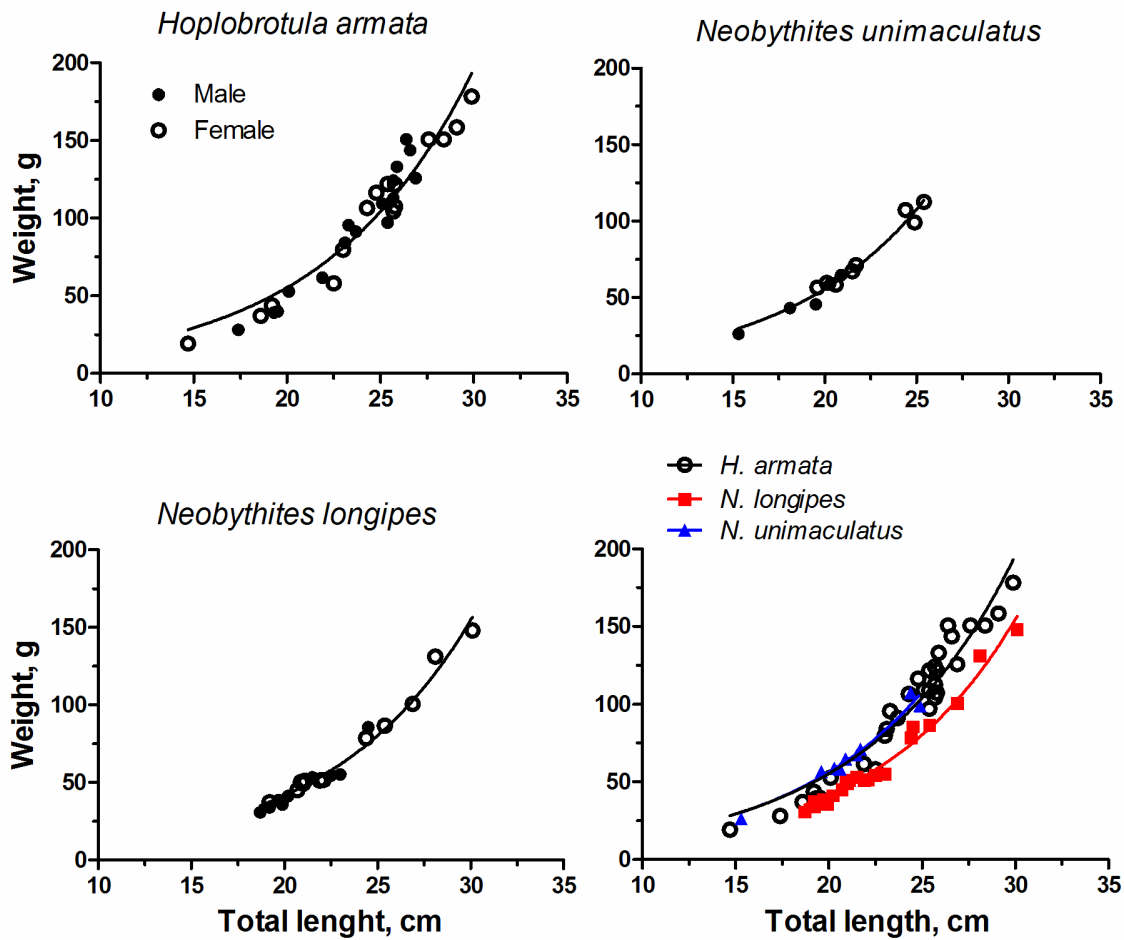


Figure 3 **A** Relationship of weight to length for combined males and females in *Hoplobrotula armata*: $Y = 4.412e^{0.126x}$, $r^2 = 0.93$. **B** Relationship of weight to length for combined males and females in *Neobythites longipes*: $Y = 3.334e^{0.127x}$, $r^2 = 0.97$. **C** Relationship of weight to length for combined males and females in *Neobythites unimaculatus*: $Y = 3.961e^{0.132x}$, $r^2 = 0.97$. **D** Three species plotted on the same graph.

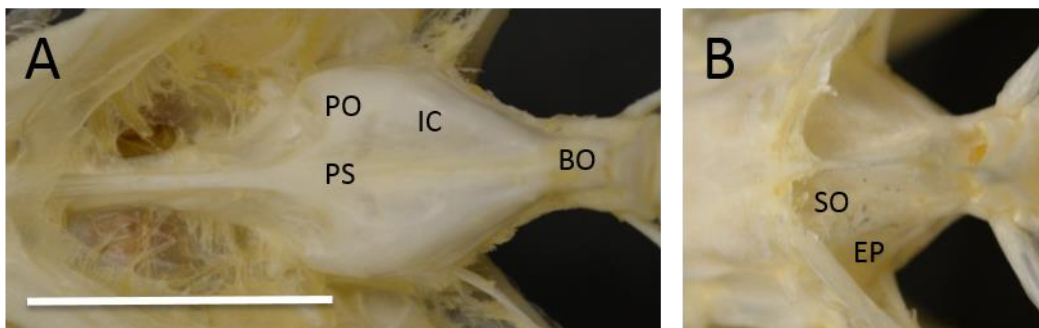


Figure 4 Photograph of the skeleton illustrating sonic muscle sites of origins of *Hoplobrotula armata*. **A.** Ventral surface illustrating origins of the ventral muscle on the prootic, the medial intermediate muscles on the border of the prootic and intercalarium. **B.** Dorsal view showing the origin of the lateral muscle and lateral-intermediate muscle on the epiotic. Scale bar = 1cm.

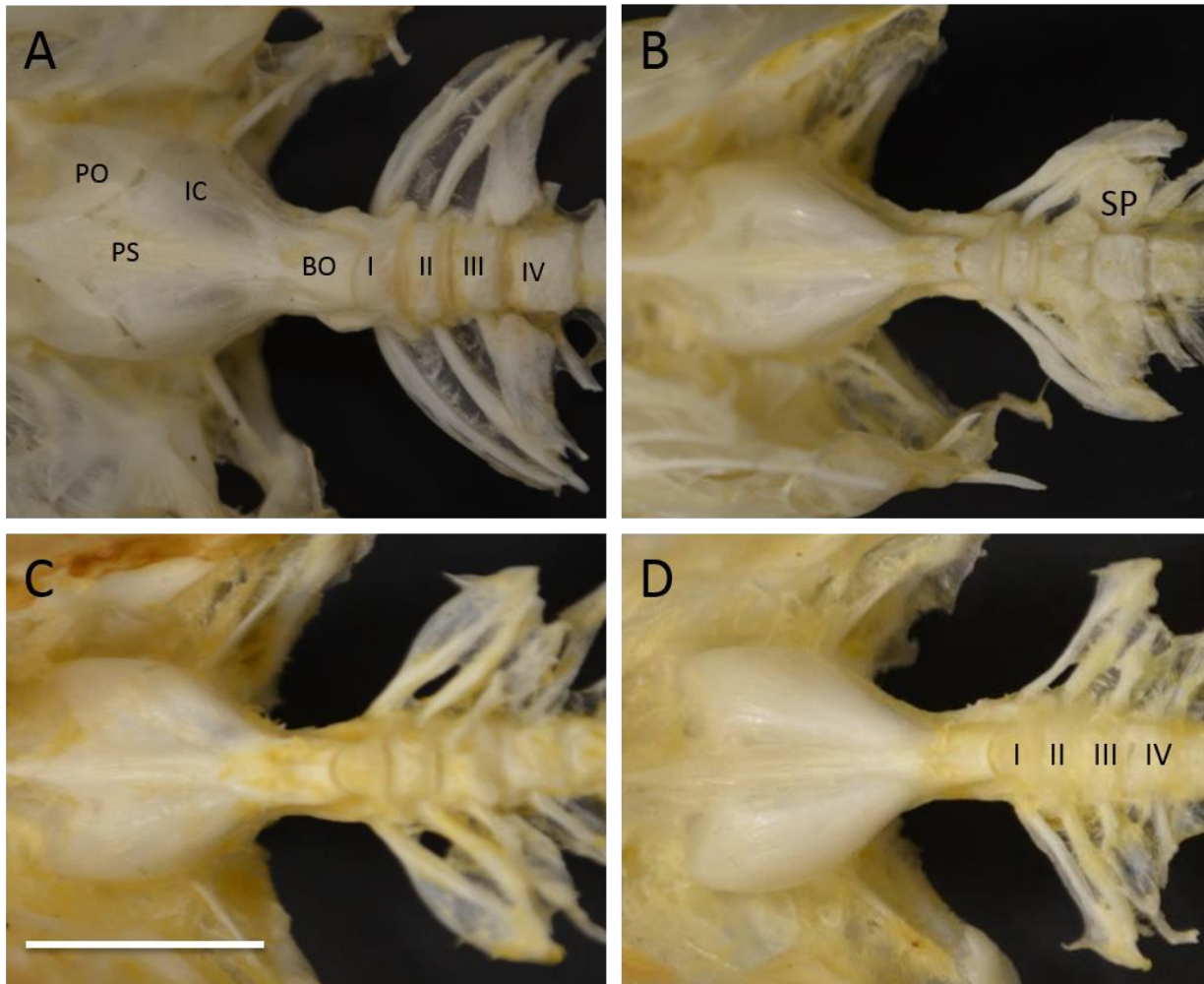


Figure 5 Photograph of the skeleton illustrating sonic muscle origins and differences in epineural ribs. **A.** Ventral surface of *Hoplobrotula armata* illustrating origins of the ventral muscle on the prootic, the medial intermediate muscles on the border of the prootic and interclarium, and the epineural ribs on the first three vertebra and the swimbladder plate. **B.** Ventral view of the epineural ribs in *Neobythites longipes*. Note the smaller swimbladder plate. **C.** and **D.** Epineural ribs of female and male *Neobythites unimaculatus* illustrating sexual differences. The swimbladder plate is further reduced. Abbreviations: BO basioccipital, EP epiotic, IC interclarium, PO prootic, PS parasphenoid, SO supraoccipital, SP swimbladder plate. Scale bar = 1cm.

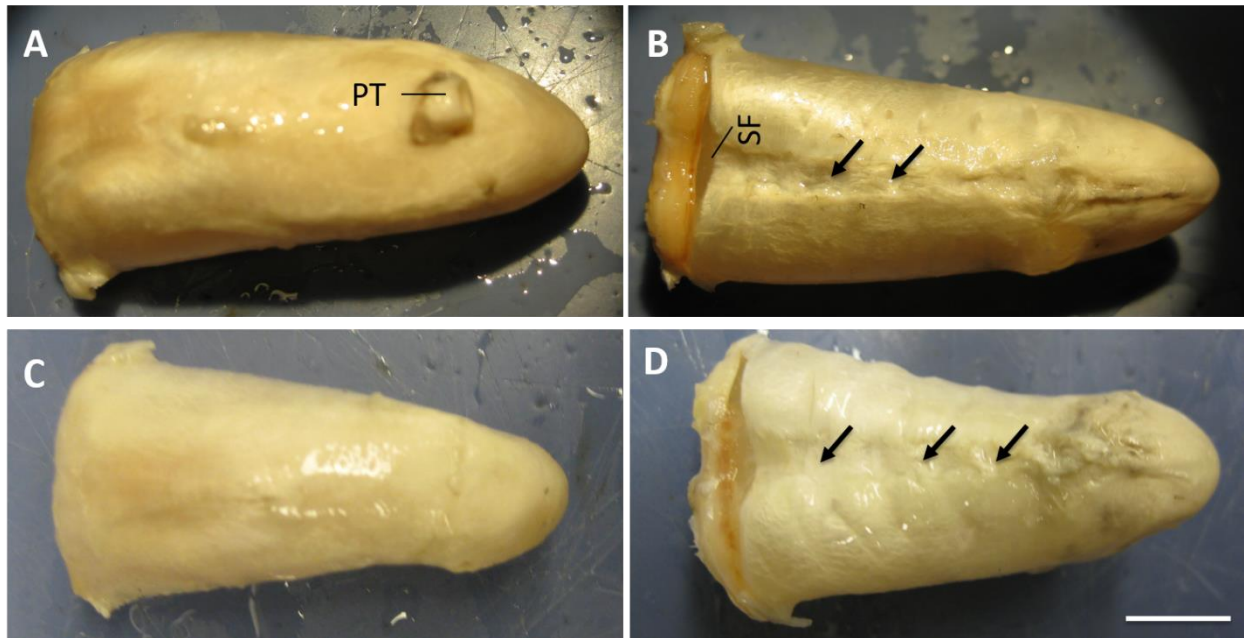


Figure 6 Photograph of the swimbladder in *Hoplobrotula armata*. **A.** Ventral view in a male illustrating the posterior tube (PT). **C.** Ventral view of the swimbladder in a female. **B.** and **D.** Dorsal view in male and female showing the swimbladder fenestra (SF) and attachments to the vertebral column. Scale bar = 1cm.

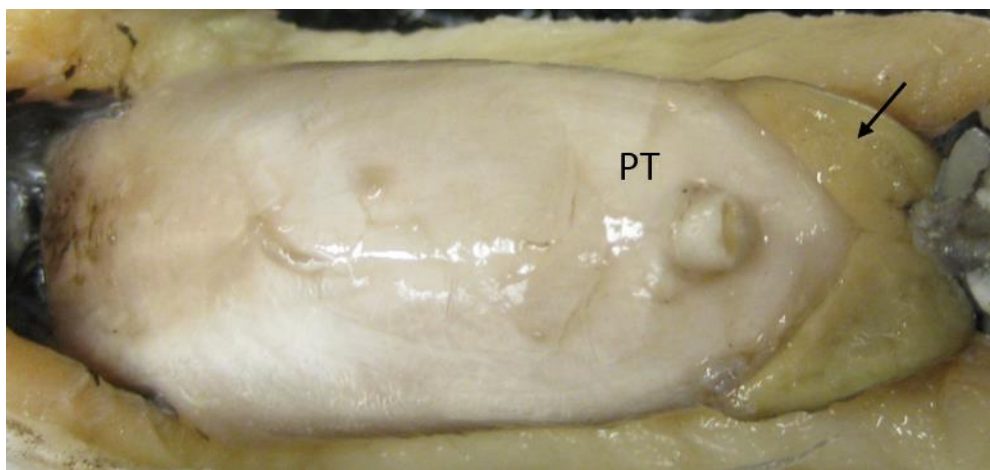


Figure 7 Photograph of the swimbladder in *Hoplobrotula armata*. Ventral view illustrating the posterior tube (PT) and concave soft-tissue structure (arrow) caudal to the swimbladder.

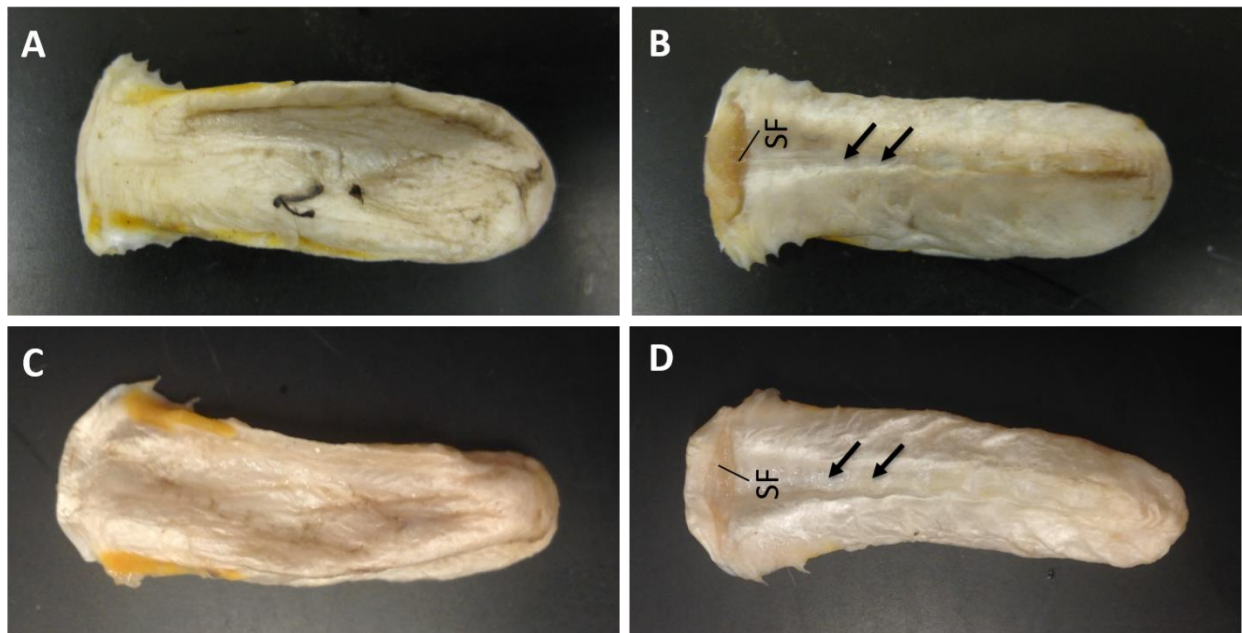


Figure 8 Photograph of the swimbladder in *Neobythites longipes*. **A.** and **C.** Ventral view in a male (A) and female (C). **B.** and **D.** Dorsal view in male (B) and female (D) showing the swimbladder fenestra (SF), and attachments to the vertebral column. The fenestra is longer in female.

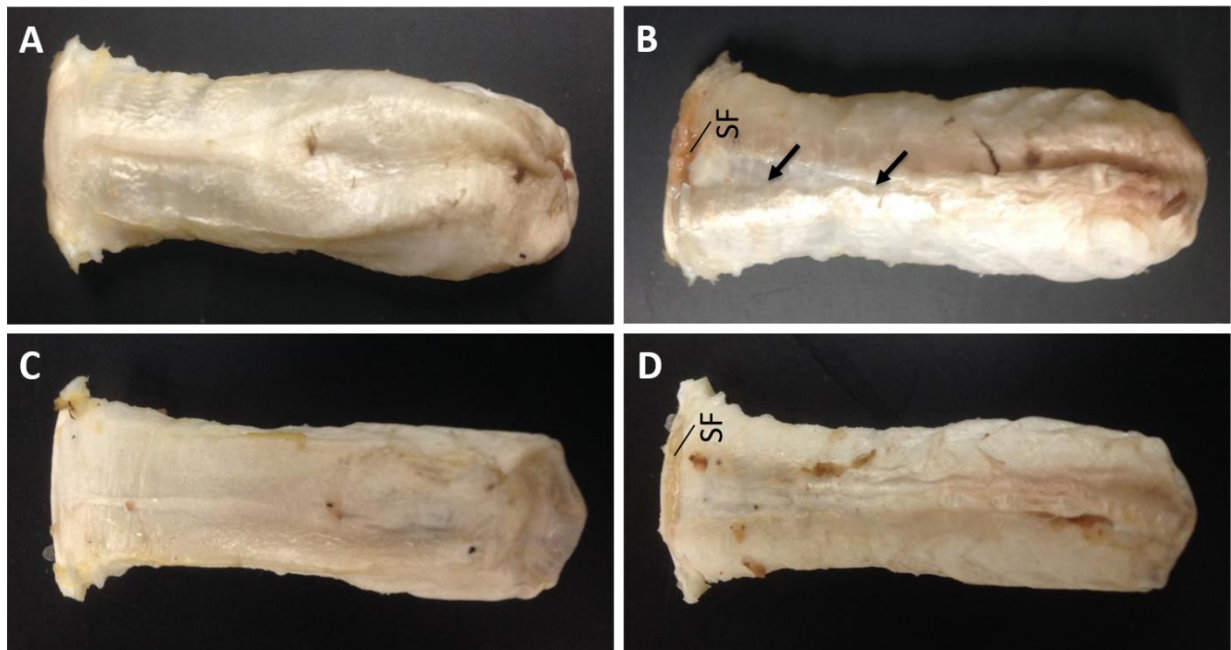


Figure 9 Photograph of the swimbladder in *Neobythites unimaculatus*. **A.** and **C.** Ventral view in male (A) and female (C). **B.** and **D.** Dorsal view in male (B) and female (D) showing the swimbladder fenestra (SF) and attachments to the vertebral column.

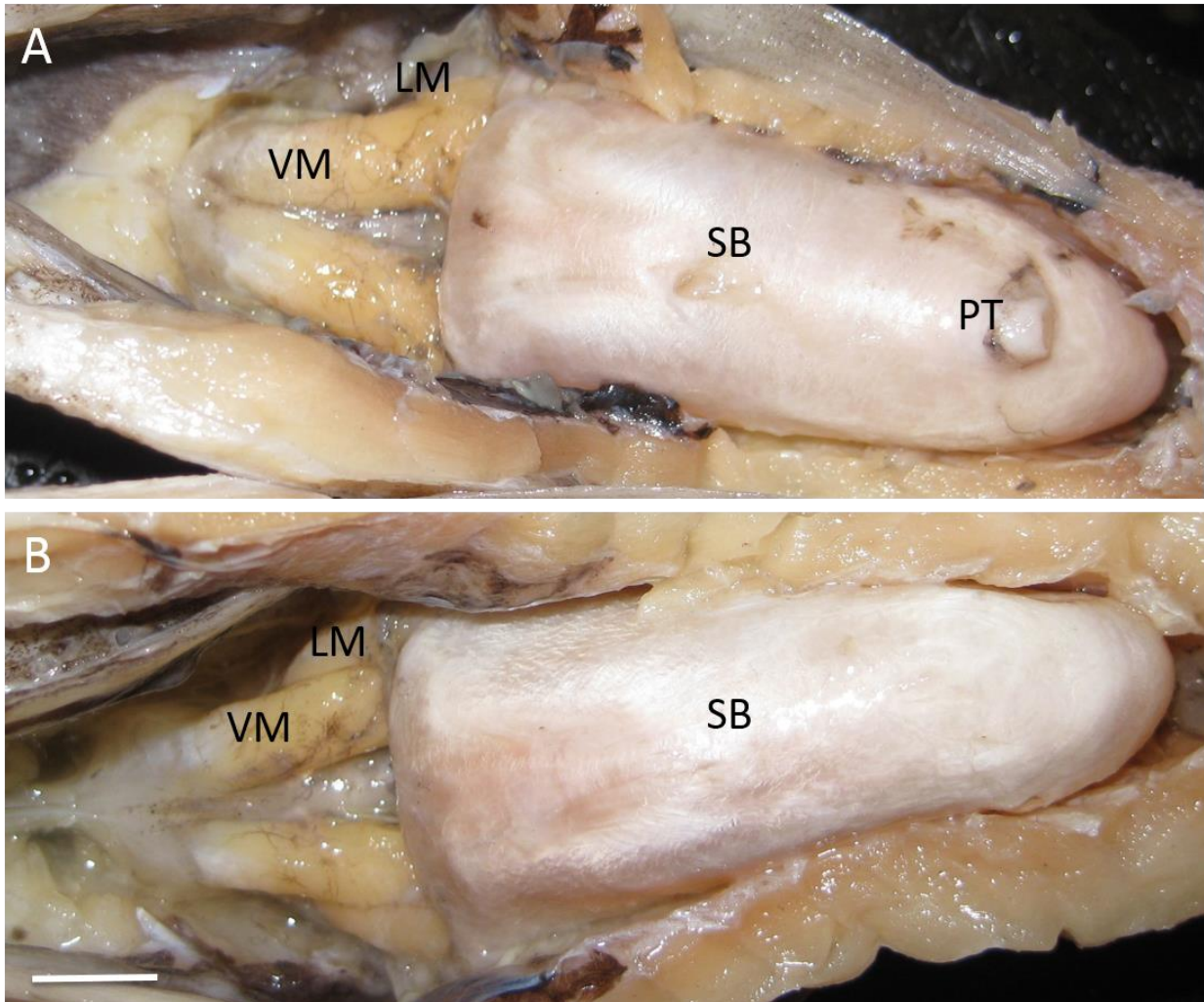


Figure 10 Photograph of the sonic muscles and swimbladder in *Hoplobrotula armata*. **A.** Ventral view in male illustrating the large ventral muscles (VM), lateral muscle (LM) and the posterior tube (PT) on the swimbladder. **B.** Ventral view in female illustrating the ventral muscles and the large lateral muscles. Scale bar = 1cm.

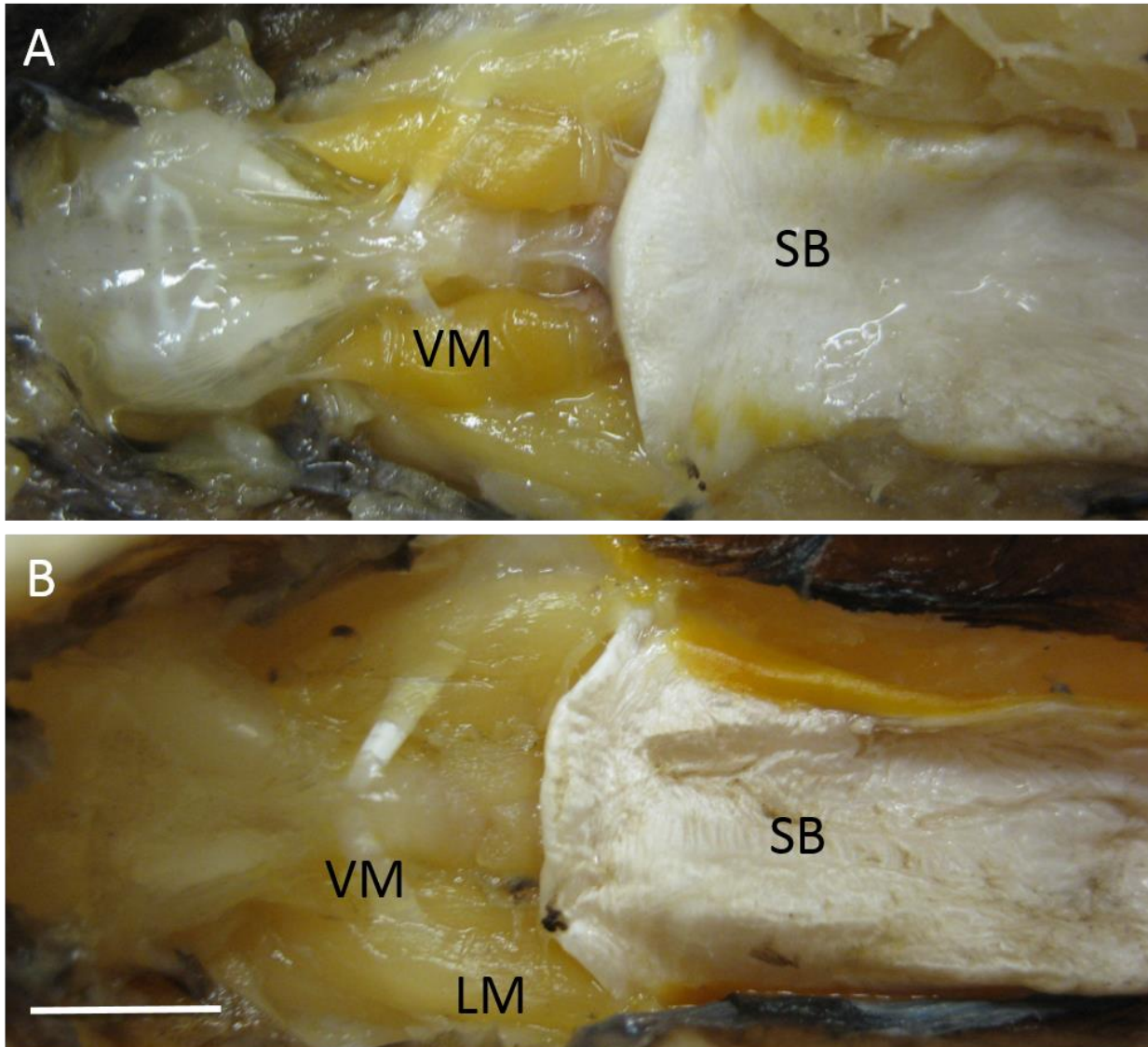


Figure 11 Photograph of the sonic muscles and swimbladder in *Neobythites longipes*. **A.** Ventral view in male illustrating the large ventral muscles (VM), lateral muscle (LM). **B.** Ventral view in female illustrating the ventral muscles and the large lateral muscles. Scale bar = 1cm.

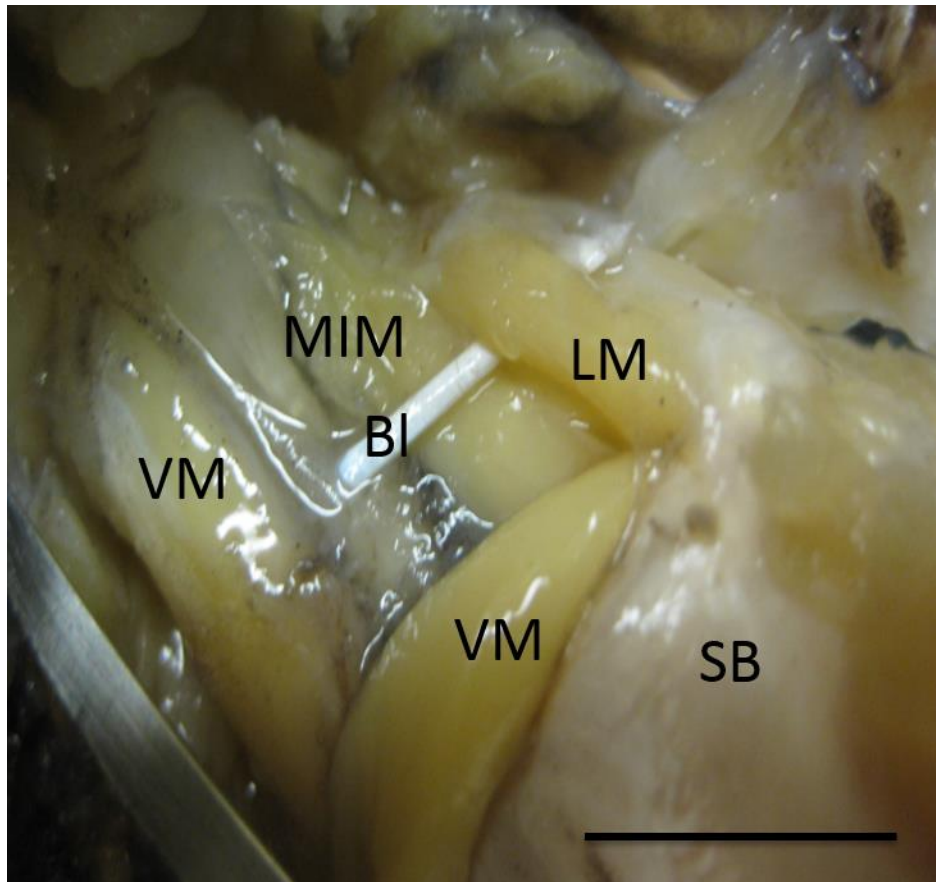


Figure 12 Sonic muscles in *Hoplobrotula armata*. The ventral and lateral muscles have been removed on the left side to expose the medial intermediate and lateral intermediate muscles that lie above Baudelot's ligament. Abbreviations: BL baudelot's ligament, MIM medial intermediate muscle, LIM lateral intermediate muscle, LM lateral muscle, SB swimbladder, VM ventral muscle. Scale bar = 1cm.

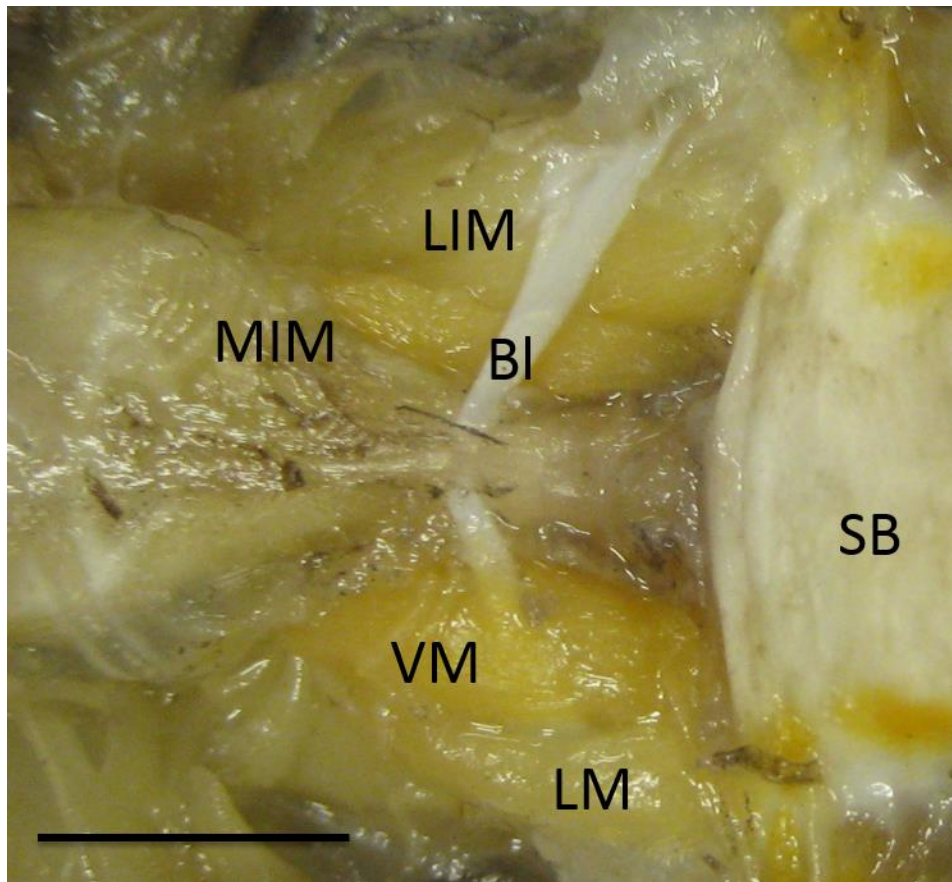


Figure 13 Sonic muscles in *Neobythites longipes*. The ventral and lateral muscles have been removed on the left side to expose the medial intermediate and lateral intermediate muscles that lie above Baudelot's ligament. Abbreviations: BL baudelot's ligament, MIM medial intermediate muscle, LIM lateral intermediate muscle, LM lateral muscle, SB swimbladder, VM ventral muscle. Scale bar = 1cm.

Hoplobrotula armata

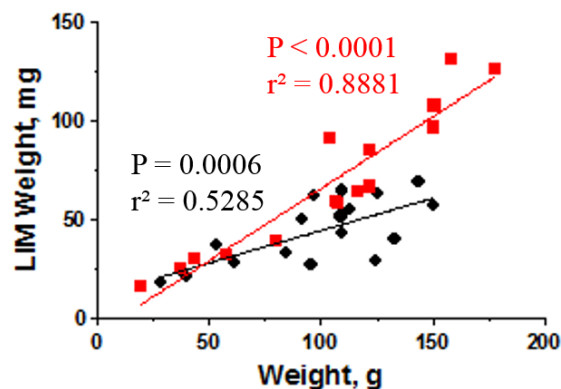
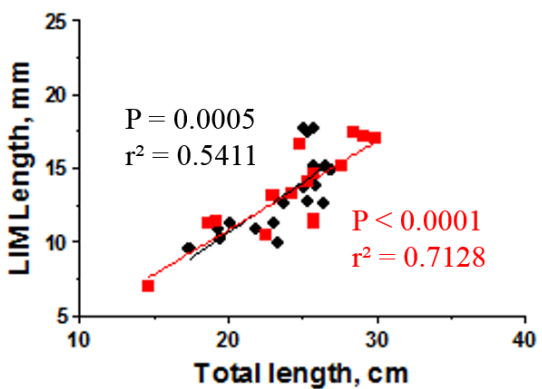
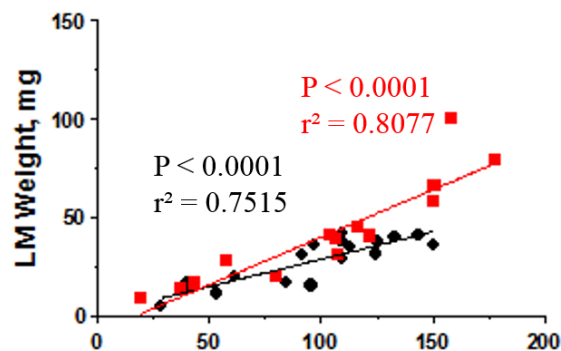
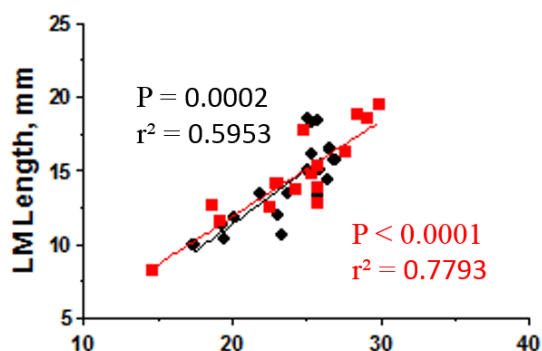
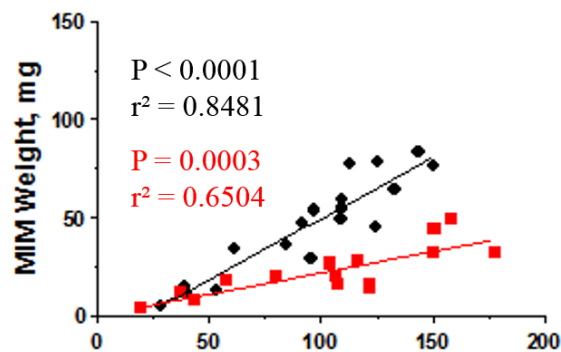
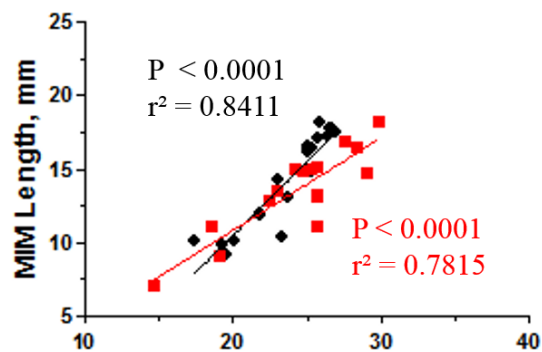
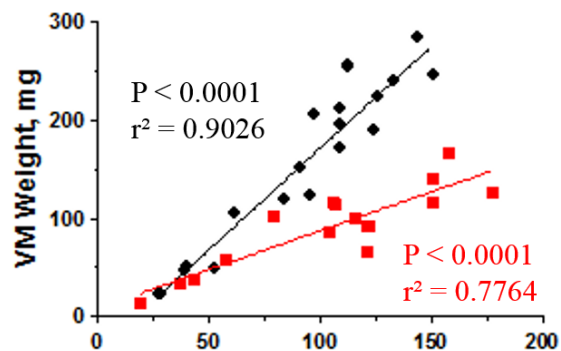
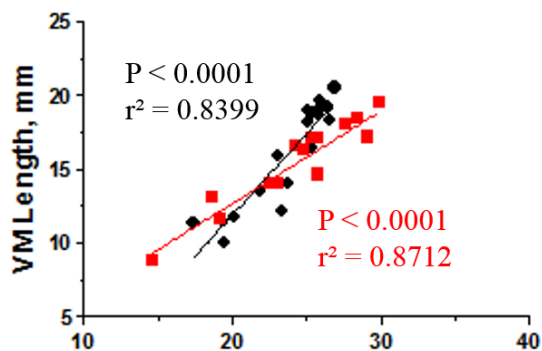


Figure 14 Relationship of length and weight of sonic muscles to fish total length (left column) and weight (right column), r^2 and p values for the ventral, medial intermediate, lateral and lateral intermediate muscles in *Hoplobrotula armata*.

Neobythites longipes

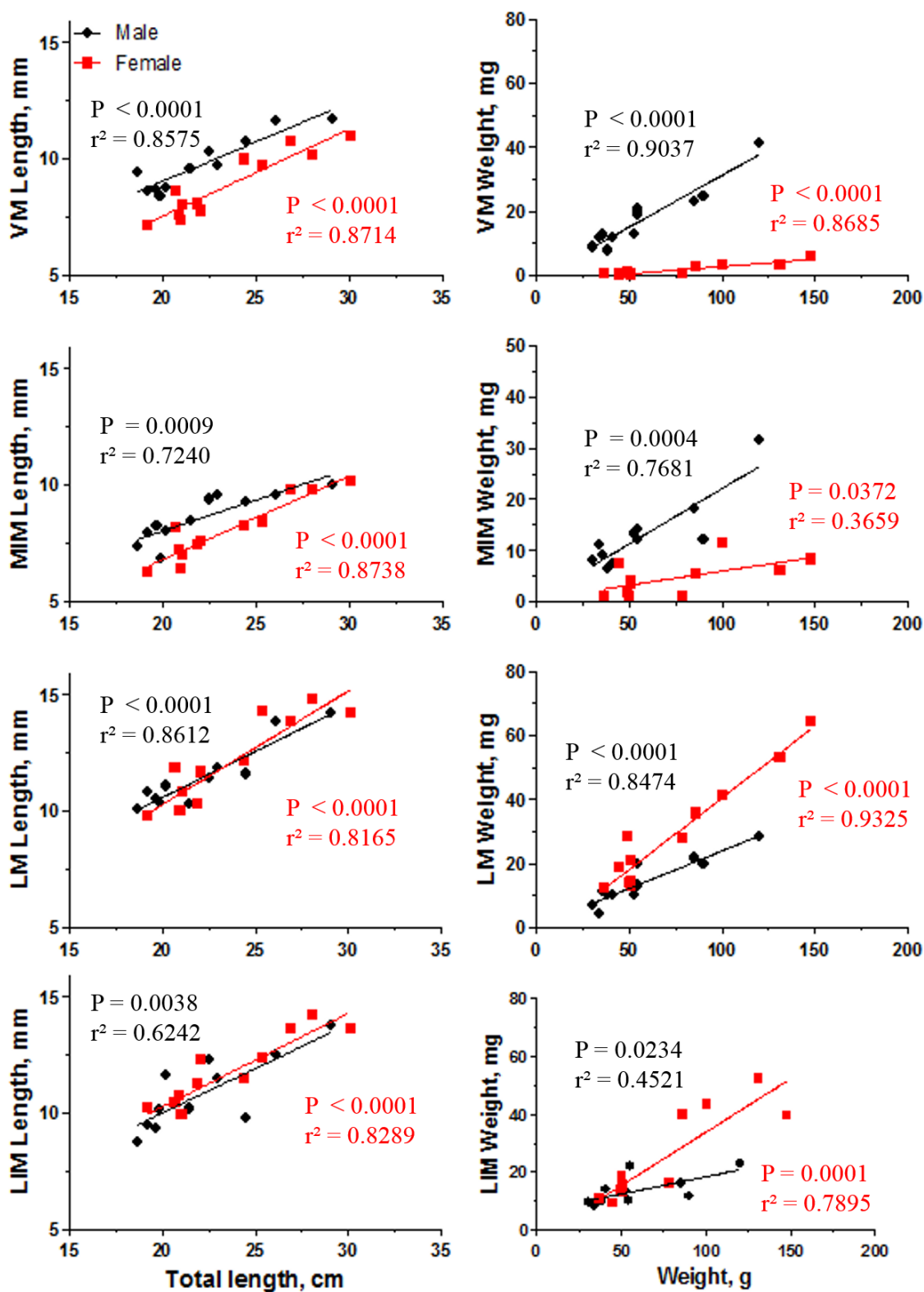


Figure 15 Relationship of length and weight of sonic muscles to fish total length (left column) and weight (right column), r^2 and p values for the ventral, medial intermediate, lateral and lateral intermediate muscles in *Neobythites longipes*.

Neobythites unimaculatus

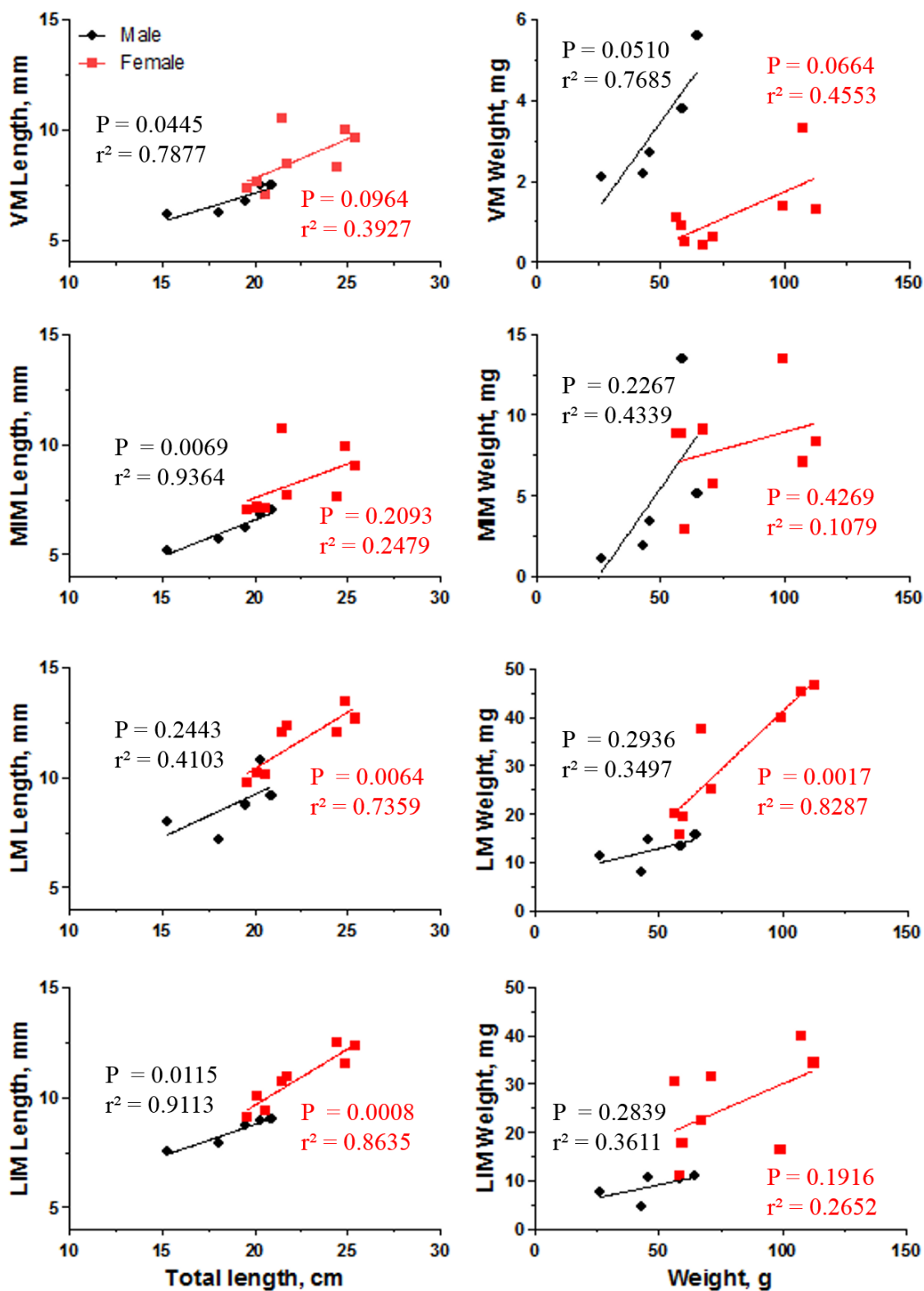


Figure 16 Relationship of length and weight of sonic muscles to fish total length (left column) and weight (right column), r^2 and p values for the ventral, medial intermediate, lateral and lateral intermediate muscles in *Neobythites unimaculatus*.

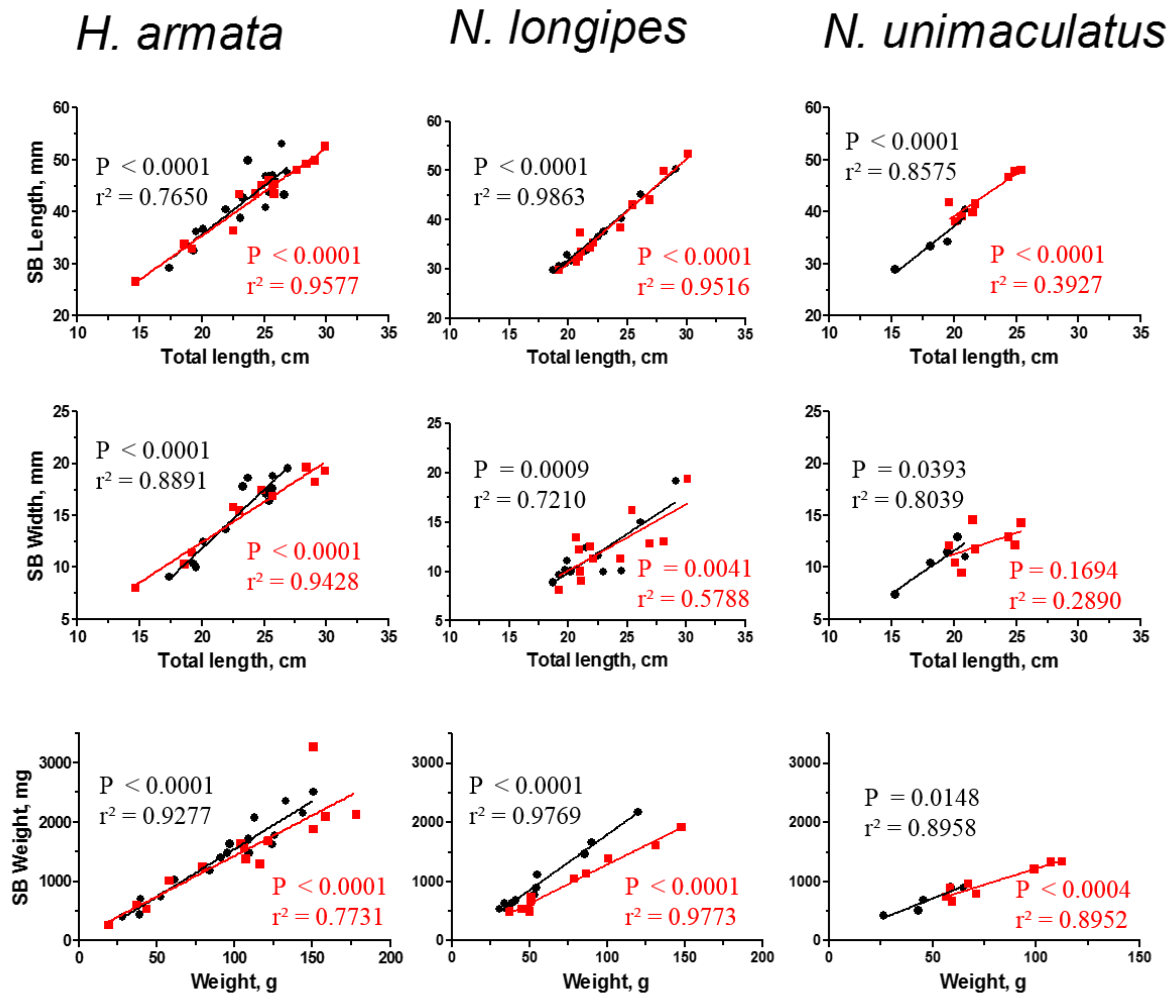


Figure 17 Relationship of swimbladder length (top row) and width (middle row) to fish total length and swimbladder weight (bottom row) to fish weight, r^2 and p values for male and female *Hoplobrotula armata*, *Neobythites longipes* and *Neobythites unimaculatus*.

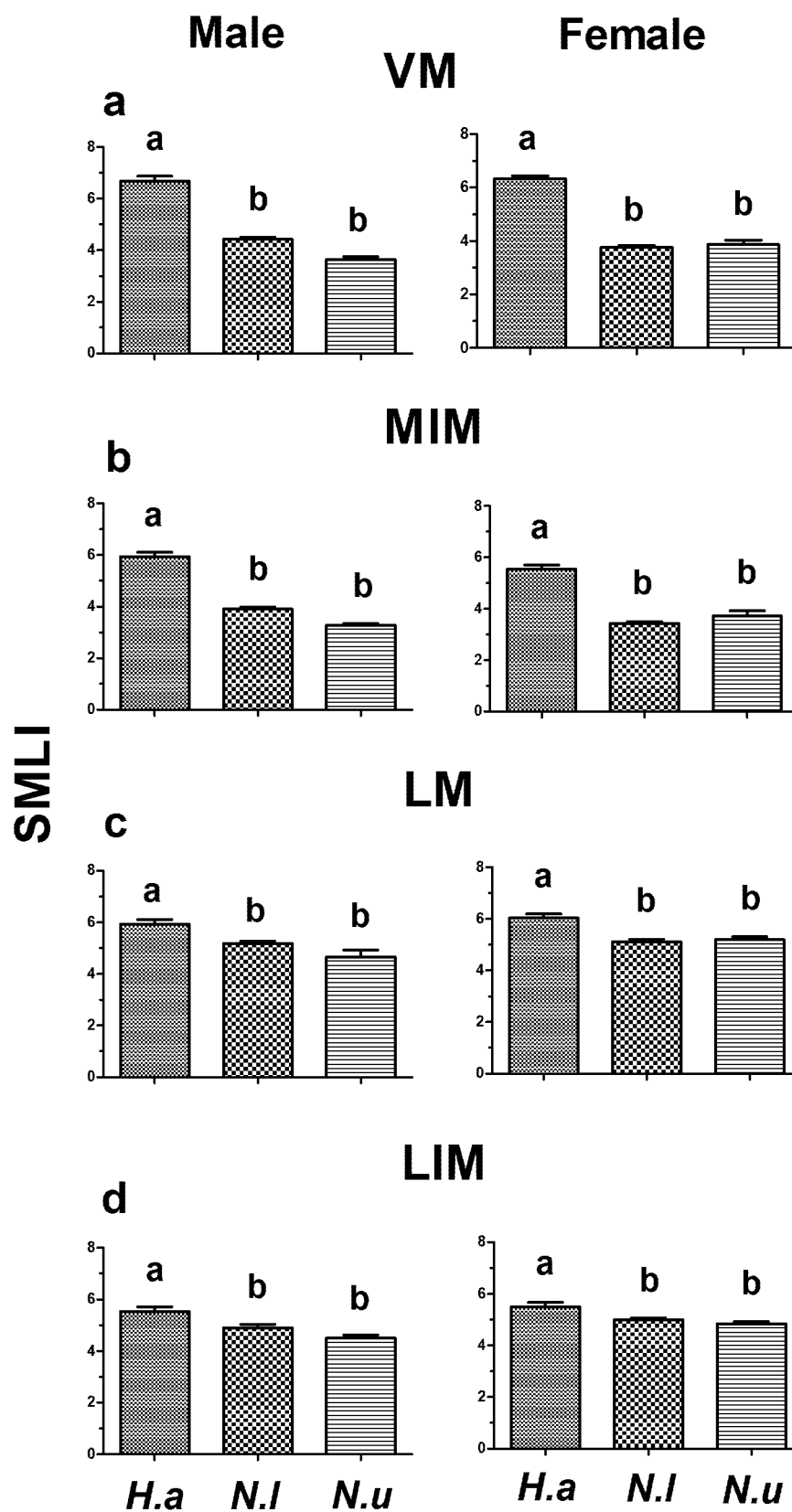


Figure 18 Sonic muscle length index $[(\text{sonic muscle length} \times \text{TL}^{-1})100]$ for male and female *Hoplobrotula armata*, *Neobythites longipes* and *Neobythites unimaculatus*. Different letters indicate significant differences.

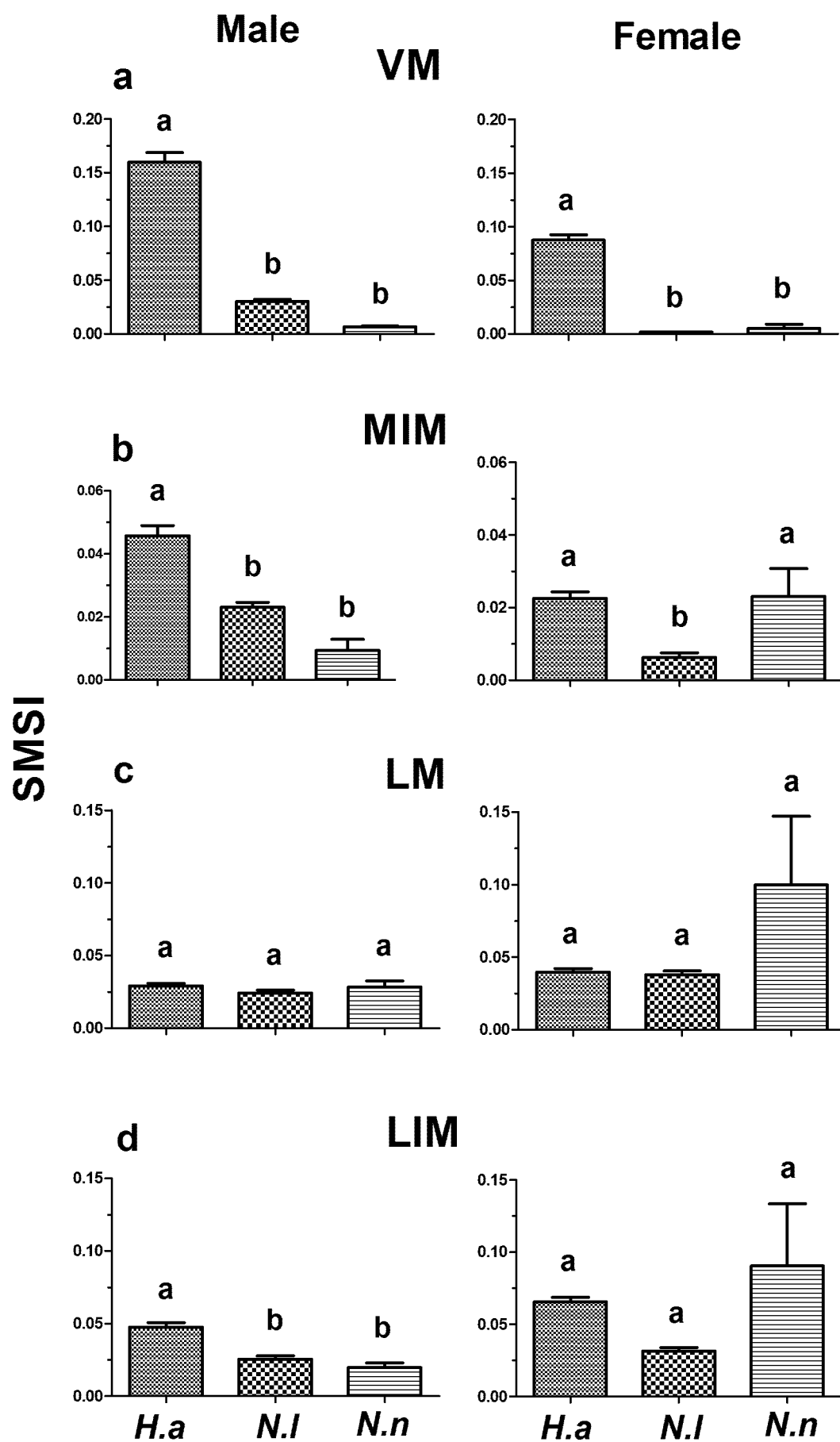
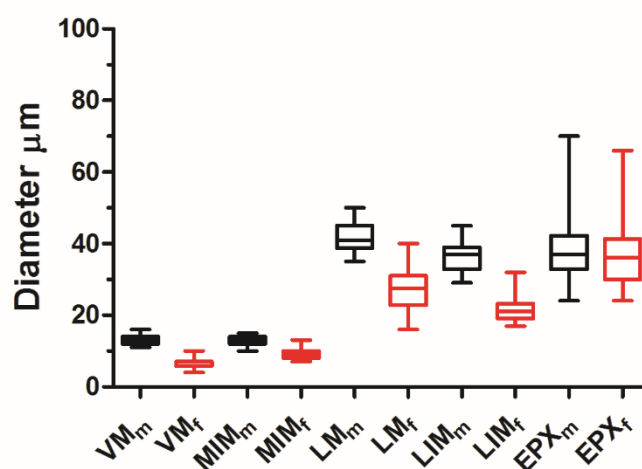
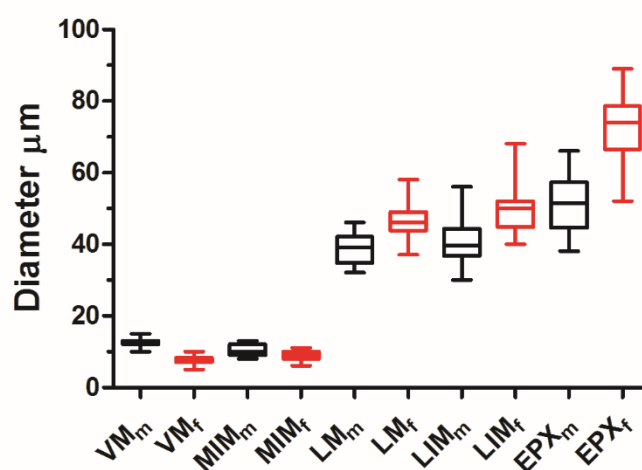


Figure 19 Sonic muscle somatic index $[(\text{sonic muscle weight} \times \text{Wt}^{-1})100]$ for male and female *Hoplobrotula armata*, *Neobythites longipes* and *Neobythites unimaculatus*. Different letters indicate significant differences.

Hoplobrotula armata



Neobythites longipes



Neobythites unimaculatus

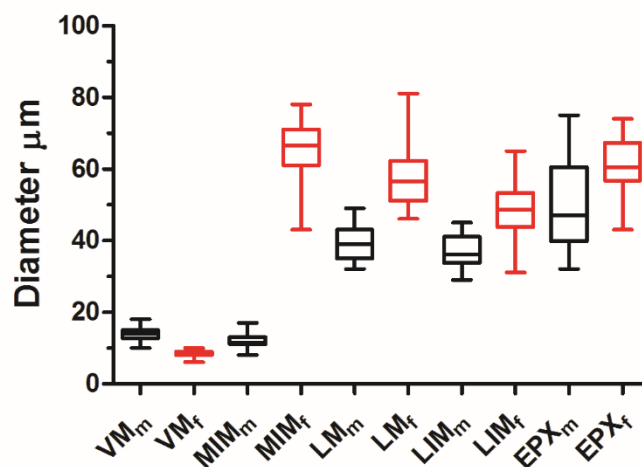


Figure 20 Box and whiskers plot of minimal fiber diameter for the ventral, medial intermediate, lateral and lateral intermediate muscles of male and female *Hoplbrotula armata* (Male TL 26.2 cm, 143.7 g, Female TL 28.4 cm, 150.8 g), *Neobythites longipes* (Male TL 23.0 cm, 55.2 g, Female TL 25.4 cm, 86.5 g) and *N. unimaculatus* (Male TL 19.5 cm, 45.5 g, Female TL 19.6 cm, 56.4 g).

References

Angel, M.V., 1997. What is the deep sea. In: Randall, D.J., Farrell, A.P. (Eds.), Deep-Sea Fishes. Academic Press, New York, pp. 1-41.

Appelt, D., Shen, V., Franzini-Armstrong, C., 1991. Quantitation of Ca ATPase, feet and mitochondria in superfast muscle fibres from the toadfish, *Opsanus tau*. Journal of Muscle Research & Cell Motility 12 (6), 543-552.

Bochus, A.B., Seibel, B.A., 2016. Trimethylamine oxide accumulation as a function of depth in Hawaiian mid-water fishes. Deep-Sea Research I 112,37-44.

Carter, H.J., Musick, J.A., 1985. Sexual dimorphism in the deep-sea fish *Barathrodemus manatinus* (Ophidiidae). Copeia (1), 69-73.

Connaughton, M.A., 2004. Sound generation in the searobin (*Prionotus carolinus*), a fish with alternate sonic muscle contraction. Journal of Experimental Biology 207 (10), 1643-1654.

Connaughton, M.A., Taylor, M.H., Fine, M.L., 2000. Effects of fish size and temperature on weakfish disturbance calls: implications for the mechanism of sound generation. Journal of Experimental Biology 203 (9), 1503-1512.

Courtenay, W., 1971. Sexual dimorphism of sound producing mechanism of striped cusk-eel, *rissola-marginata* (Pisces: Ophidiidae). Copeia (2), 259-268.

Fine, M.L., 2012. Swimbladder sound production: the forced response versus the resonant bubble. Bioacoustics-the International Journal of Animal Sound and Its Recording 21 (1), 5-7.

Fine, M.L., Bernard, B., Harris, T.M., 1993. Functional-morphology of toadfish sonic muscle-fibers - relationship to possible fiber division. Canadian Journal of Zoology 71 (11), 2262-2274.

Fine, M.L., Burns, N.M., Harris, T.M., 1990. Ontogeny and sexual dimorphism of sonic muscle in the oyster toadfish. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 68 (7), 1374-1381.

Fine, M.L., Lin, H., Nguyen, B.B., Rountree, R.A., Cameron, T.M., Parmentier, E., 2007. Functional morphology of the sonic apparatus in the fawn cusk-eel *Lepophidium profundorum* (Gill, 1863). *Journal of Morphology* 268 (11), 953-966.

Fine, M.L., Malloy, K.L., King, C.B., Mitchell, S.L., Cameron, T.M., 2001. Movement and sound generation by the toadfish swimbladder. *Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology* 187 (5), 371-379.

Fine, M.L., Parmentier, E., 2015. Mechanisms of fish sound production. In: Ladich, F. (Ed.), *Sound communication in fishes*. Springer Verlag, Wien., pp. 77-126.

Gartner Jr, J.V., 1997. Feeding at depth. In: Randall, D.J., R., Farrell, A.P. (Eds.), *Deep-Sea Fishes*. Academic Press, New York, pp. 115-193.

Hill, G.L., Fine, M.L., Musick, J.A., 1987. Ontogeny of the sexually dimorphic sonic muscle in 3 sciaenid species. *Copeia* (3), 708-713.

Iwamoto, T., McCosker, J., 2014. Deep-water fishes of the 2011 Hearst Philippine biodiversity expedition of the California Academy of Sciences. In: GC Gosliner, G.C., Williams, T.M. (Eds.). *The coral triangle*, San Francisco pp. 263 - 332.

Kever, L., Boyle, K.S., Bolen, G., Dragicevic, B., Dulcic, J., Parmentier, E., 2014a. Modifications in Call Characteristics and Sonic Apparatus Morphology During Puberty in *Ophidion rochei* (Actinopterygii: Ophidiidae). *Journal of Morphology* 275 (6), 650-660.

Kever, L., Boyle, K.S., Dragicevic, B., Dulcic, J., Casadevall, M., Parmentier, E., 2012. Sexual dimorphism of sonic apparatus and extreme intersexual variation of sounds in *Ophidion rochei* (Ophidiidae): first evidence of a tight relationship between morphology and sound characteristics in Ophidiidae. *Frontiers in Zoology* 9.

Kever, L., Boyle, K.S., Dragicevic, B., Dulcic, J., Parmentier, E., 2014b. A superfast muscle in the complex sonic apparatus of *Ophidion rochei* (Ophidiiformes): histological and physiological approaches. *Journal of Experimental Biology* 217 (19), 3432-3440.

Ladich, F., Fine, M.L., 2006. Sound-generating mechanisms in fishes: a unique diversity in vertebrates. In: Ladich F, C.S., Moller P, Kapoor BG. (Ed.), *Communication in fishes*. Science Publisher, Enfield, NH, pp. 3-34.

Mann, D.A., Bowers-Altman, J., Rountree, R.A., 1997. Sounds produced by the striped cusk-eel *Ophidion marginatum* (Ophidiidae) during courtship and spawning. *Copeia* 1997 (3), 610-612.

Mann, D.A., Jarvis, S.M., 2004. Potential sound production by a deep-sea fish. *The Journal of the Acoustical Society of America* 115 (5), 2331-2333.

Marshall, N.B., 1962. The biology of sound-producing fishes. *Symp. Zool. Soc. Lond*, pp. 45-60.

Marshall, N.B., 1967. Sound-producing mechanisms and the biology of deep-sea fishes. In: Tavalga, W.N. (Ed.), *Marine bio-acoustics*. Pergamon Press, New York, pp. 123-133.

Masuda, H., Amaoka, K., Araga, C., Uyeno, T., Yoshino, T., 1984. *The Fishes of the Japanese Archipelago*, (plates) Tokai University Press, Shinjuku Tokai Building. Tokyo, Japan.

- Millot, S., Vandewalle, P., Parmentier, E., 2011. Sound production in red-bellied piranhas (*Pygocentrus nattereri*, Kner): an acoustical, behavioural and morphofunctional study. *The Journal of experimental biology* 214 (21), 3613-3618.
- Mok, H.-K., Parmentier, E., Chiu, K.-H., Tsai, K.-E., Chiu, P.-H., Fine, M.L., 2011. An Intermediate in the evolution of superfast sonic muscles. *Frontiers in Zoology* 8.
- Mooney, T.A., Kaplan, M.B., Annamaria, I., Luca, L., Laela, S., 2016. Temporal trends in cusk eel sound production at a proposed US wind farm site. *Aquatic Biology* 24, 201-210.
- Nguyen, T.K., Lin, H., Parmentier, E., Fine, M.L., 2008. Seasonal variation in sonic muscles in the fawn cusk-eel *Lepophidium profundorum*. *Biology Letters* 4 (6), 707-710.
- Nielsen, J.G., Cohen, D.M., Markle, D.F., Robins, C.R., 1999. FAO species catalogue. Ophidiiform fishes of the world (Order Ophidiiformes). An annotated and illustrated catalogue of pearlfishes, cusk-eels, brotulas and other ophidiiform fishes known to date. FAO Fisheries Synopsis 125.
- Parmentier, E., Bouillac, G., Dragicevic, B., Dulcic, J., Fine, M., 2010. Call properties and morphology of the sound-producing organ in *Ophidion rochei* (Ophidiidae). *Journal of Experimental Biology* 213 (18), 3230-3236.
- Parmentier, E., Diogo, R., 2006. Evolutionary trends of swimbladder sound mechanisms in some teleost fishes. *Communication in fishes* 1, 43-68.
- Parmentier, E., Fine, M.L., Mok, H.K., 2016. Sound production by a recoiling system in the Pempheridae and Terapontidae. *Journal of Morphology* in press.

- Parmentier, E., Fontenelle, N., Fine, M.L., Vandewalle, P., Henrist, C., 2006a. Functional morphology of the sonic apparatus in *Ophidion barbatum* (Teleostei, Ophidiidae). *Journal of Morphology* 267 (12), 1461-1468.
- Parmentier, E., Lagardere, J.-P., Braquegnier, J.-B., Vandewalle, P., Fine, M.L., 2006b. Sound production mechanism in carapid fish: first example with a slow sonic muscle. *Journal of Experimental Biology* 209 (15), 2952-2960.
- Rome, L.C., Lindstedt, S.L., 1998. The quest for speed: muscles built for high-frequency contractions. *Physiology* 13 (6), 261-268.
- Rose, J.A., 1961. Anatomy and sexual dimorphism of the swim bladder and vertebral column in *Ophidion holbrooki* (Pisces: Ophidiidae). *Bulletin of Marine Science* 11 (2), 280-308.
- Rountree, R.A., Bowers-Altman, J., 2002. Soniferous behaviour of the striped cusk-eel *Ophidion marginatum*. *Bioacoustics* 12 (2-3), 240-242.
- Rountree, R.A., Juanes, F., 2010. First attempt to use a remotely operated vehicle to observe soniferous fish behavior in the Gulf of Maine, Western Atlantic Ocean. *Current Zoology* 56 (1), 90-99.
- Rountree, R.A., Juanes, F., Goudey, C.A., Ekstrom, K.E., 2012. Is biological sound production important in the deep sea? In: Popper, A.N., Hawkins, A. (Eds.), *The Effects of Noise on Aquatic Life*. Springer, pp. 181-183.
- Skoglund, C.R., 1961. Functional analysis of swim-bladder muscles engaged in sound production of the toadfish. *Journal of Biophysical and Biochemical Cytology* 10 (Supple), 187-200.

- Sprague, M.W., Luczkovich, J.J., Schaefer, S.A., 2001. Do striped cusk-eels *Ophidion marginatum* (Ophidiidae) produce the "chatter" sound attributed to weakfish *Cynoscion regalis* (Sciaenidae)? *Copeia* 2001 (3), 854-859.
- Sutton, T.T., Wiebe, P.H., Madin, L., Bucklin, A., 2010. Diversity and community structure of pelagic fishes to 5000m depth in the Sargasso Sea. *Deep Sea Research Part II: Topical Studies in Oceanography* 57 (24), 2220-2233.
- Wall, C.C., Lembke, C., Mann, D.A., 2012. Shelf-scale mapping of sound production by fishes in the eastern Gulf of Mexico, using autonomous glider technology. *Marine Ecology Progress Series* 449, 55-64.
- Wall, C.C., Rountree, R.A., Pomerleau, C., Juanes, F., 2014. An exploration for deep-sea fish sounds off Vancouver Island from the NEPTUNE Canada ocean observing system. *Deep Sea Research Part I: Oceanographic Research Papers* 83, 57-64.
- Wall, C.C., Simard, P., Lembke, C., Mann, D.A., 2013. Large-scale passive acoustic monitoring of fish sound production on the West Florida Shelf. *Mar Ecol Prog Ser* 484, 173-188.

